BIOPOTENCY OF INDIAN PRIVET, *Vitex negundo* Linn. (Verbenaceae) AGAINST *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* Fab. (Coleoptera: Coccinellidae)

By

DEEPTHY. K.B

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Biopotency of Indian privet, *Vitex negundo* Linn. (Verbenaceae) against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* Fab. (Coleoptera: Coccinellidae)"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara Deepthy.K.B.

CERTIFICATE

Certified that this thesis entitled **"Biopotency of Indian privet, *Vitex negundo* Linn. (Verbenaceae) against *Spodoptera litura* Fab.(Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* Fab.(Coleoptera: Coccinellidae)"** is a bonafide record of research work done independently by **Miss. Deepthy.K.B.** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to her.

VELLANIKKARA

DR. M.K. SHEELA,

(Major Advisor, Advisory Committee)
Director of Extension,
Kerala Agricultural University

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Miss. Deepthy. K.B.**, a candidate for the degree of **Doctor of Philosophy in Agriculture** with major in **Agricultural Entomology**, agree that this thesis entitled "**Biopotency of Indian privet, *Vitex negundo* L. (Verbenaceae) against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* Fab. (Coleoptera: Coccinellidae)**" may be submitted by **Miss. Deepthy. K.B.**, in partial fulfillment of the requirement for the degree.

Dr. M.K. Sheela
(Chairperson, Advisory committee)
Director of Extension
Kerala Agricultural University

Dr. Sosamma Jacob
(Member, Advisory committee)
Professor and Head
Department of Agricultural Entomology
College of Horticulture
Vellanikkara

Dr. Jim Thomas
(Member, Advisory Committee)
Professor
Department of Agricultural Entomology
College of Horticulture
Vellanikkara

Dr. A. Augustin
(Member, Advisory Committee)
Professor and Associate Director of Research
Kerala Agricultural University

Dr. S. Estelitta
(Member, Advisory Committee)
Professor,
Communication centre,
Mannuthy

EXTERNAL EXAMINER

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Introduction

INTRODUCTION

Large scale use of synthetic and broad spectrum insecticides has resulted in hazardous effects on environment and human health, resistance development in insect populations, resurgence of pest populations and so forth (Pimental *et al*., 1992). Extensive research is being carried out world wide to find safer, biodegradable substitutes for these synthetic insecticides. Botanical insecticides may provide alternatives to currently used synthetic insecticides because many of them are often active against a number of species and are biodegradable and hence suitable for use in integrated pest management programmes (IPM) (Schmutterer, 1990).

The plant kingdom is the most efficient chemical factory. The secondary metabolites produced by plants have a wide range of mode of actions. These compounds are deleterious to insects and other herbivores in multiple ways, such as causing acute toxicity, affecting insect behaviour, disrupting growth and development of insects, acting as repellents, deterring oviposition, being ovicidal, inhibit enzyme activity and interfering with consumption and (or) utilization of food (Wheeler and Isman, 2001; Wheeler *et al*., 2001; Nathan 2006). Over the past 30 years search for plants with novel insecticidal constituents has been intensive. Grainage *et al*. (1984) enlisted 1005 plant species with pest repellency, 27 species with phagostimulant effects and 31 species with growth inhibition properties. More than 2400 plant species have been reported to possess pesticidal properties distributed in 189 plant families (Singh, 2000).

Among the botanicals, only neem (*Azadirachta indica* Juss.) has been successfully commercialized. However, there are many promising leads from numerous plant species, which contain insecticidal compounds (Isman, 1995; Jacobson, 1989; Schmutterer,1992). Other than *A. indica*, new potential and locally available botanicals are to be exploited. *Vitex negundo* L. commonly known as Indian privet is one of the commonly available plants in Kerala all through the year and is well adapted to different agro-ecological situations. Indian privet is a deciduous shrub growing upto 3 m in height and usually prefers light

and medium soils, it can also grow well in nutritionally poor soils. This plant usually flowers from September to October, the flowers are hermophrodite, scented, pollinated by insects. This plant is also known as "Nirgundi" in Sanskrit, which means that it protects body from diseases. The leaves are astringent, febrifuge, sedative, tonic and vermifuge. *V. negundo* contains a variety of bioactive principles which are to be explored for their insect growth suppressing effects and can be effectively utilized in pest management programmes. Antifeedant property of the leaf extracts of *V. negundo* against storage pests has been reported as early as in 1928 by Chopra. *V. negundo* extracts exhibit juveno-mimetic activity against *Dysdercus cingulatus* Fab. (Saradamma *et al*., 1993) and *Spodoptera litura* Fab. larvae (Sahayaraj 1998). Besides its reported insecticidal property, it is highly valued in ayurvedic medicines and is commercially cultivated in the state.

S. litura is a polyphagous and highly destructive pest and has about 150 host species including cotton, soyabean, celery, chilli, tobacco, cotton, castor, pulses, ground nut, sunflower, banana, cabbage, tomato, other vegetables, various spice crops, ornamental plants and weeds causing heavy economic loss every year (Ramana *et al*., 1988; Rao *et al*., 1993; Gothama *et al*; 1995). This noctuid pest was first reported from the Nelson district, New zealand as a pest of tobacco (Cottier and Gourlay 1955), now it has cosmopolitan distribution, found in many parts of tropical and temperate zones. It is one of the most economically important insect pests in many countries including India, Japan, China and other countries of South east Asia (Nathan and Kalaivani, 2005; Nathan *et al*., 2005). It is an indigenous pest of a variety of crops in South Asia and was found to cause 26 - 100 per cent yield loss in ground nut (Dhir *et al*., 1992). This pest has multiple modes of attack, such as defoliator, fruit borer, nibbler, webber, skeletoniser and feeder of tender parts. During 1970's, the area under cotton and hybrids increased in Tamilnadu which facilitated in resistance build up and out break of *S. litura* during 1970's and early 80's (Dhaliwal and Arora, 2004). In recent years out breaks have been more common in South Asia mainly due to its development of insecticide resistance (Armes *et al*., 1997; Kranthi *et al*., 2001 ; Kranthi *et al*.,

2002; Ahmed *et al*.,2007; Lin *et al*.,2009) and subsequent control failures. In India, this pest has been found resistant to several insecticides (Armes *et al*., 1997; Kranthi *et al*., 2001; Kranthi *et al*.,

2002 ; Niranjan and Regupathy, 2001). This suggests that South Asian populations of *S. litura* have the potential of developing resistance to a wide range of insecticides. All the recent control failures by conventional insecticides and the frequent out breaks and pest flare up properties of this pest in India is mostly associated with the development of resistance to various insecticides. Farmers usually make several applications of synthetic insecticides for the control of this pest during the growing seasons in countries like India (Kranthi *et al*., 2002) and USA (Brewer and Trumble, 1989).

Cucurbitaceous and solanaceous vegetable crops are cultivated extensively in Kerala. Epilachna beetle (*Henosepilachna vigintioctopunctata* Fab., Coccinellidae) causes considerable economic damage to these crops due to their diverse feeding habits. It is fairly common through out India and causes considerable damage to a number of solanaceous, cucurbitaceous and leguminous crops (Alam, 1969). Both grubs and adults of *H. vigintioctopunctata* skeletonise the leaf by scraping the chlorophyll tissues, leaving the veins and vein lets intact.

The present investigation entitled “Bio potency of Indian privet *Vitex negundo* Linn. (Verbenaceae) against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* Fab. (Coleoptera : Coccinellidae) was undertaken with the following objectives:

1) To study the toxicology of different solvent extracts from leaves, shoots and flowers of *V. negundo* against *S. litura* and *H. vigintioctopunctata*
2) To test the extracts of *V. negundo* for their activity as ovipositional deterrent, ovicide, antifeedant, insect growth regulatory effects and reproductive inhibition against the test insects *S. litura* and *H. vigintioctopunctata*
3) To assess the potency of *V. negundo* extracts in combination with entomopathogens against *S. litura* and *H. vigintioctopunctata*

Review of Literature

2. REVIEW OF LITERATURE

Indiscriminate use of chemical pesticides has led to an array of problems like development of resistance in insects, resurgence of pest population and pesticide residues in the harvested products, soil, water and environment. In this situation, it is imperative to develop management strategies, which are ecofriendly, sustainable and safe to human beings and environment.

Botanicals offer good promise in pest management as they are soft, naturally occurring with very little adverse environmental impact. Secondary metabolites from plants are deleterious to insect and other herbivores in diverse ways such as causing acute toxicity, inhibits enzyme action, insect growth regulatory effect, interfering with consumption and/or utilization of food *etc*. Many botanicals as pesticides are available all over the world for the control of different harmful pests.

2.1. BIOEFFICACY OF *VITEX NEGUNDO* LINN. AGAINST INSECT PESTS

Bioefficacy of *V. negundo* against insect pests has been reported as early as in 1928 by Chopra who had observed antifeedant action against storage pests. Litsinger *et al*. (1978) observed the bioefficacy of *V. negundo* against insect pests in paddy. Rahman and Talukder (2006) reported highest F1 progeny inhibition of *Callosobruchus maculatus* F. in black gram seeds treated with acetone extracts of nishinda leaves (*V. negundo*).

V. negundo was found to be effective against the hairy caterpillar *Euproctis fraterna* M. and cotton army worm *Spodoptera litura* F. on castor (Subadrabai and Kandaswamy, 1985). Kalavathi *et al*., (1991) observed the effectiveness of *V. negundo* against *Earias vitella* F., *Diaphania indica* (Sam) and *Epilachna septima* Dieke. The bioefficacy of *V. negundo* against several pests like rice leaffolder (Sukumaran *et al*., 1989), corn weevil (Bhuiyan and Quiniones 1990), *Plutella xylostella* Linn.(Dayrit *et al*., 1995), *S. litura* (Saradamma, 1989, Sahayaraj and Sekar, 1996), and *Sitophilus zeamais* Motschulsky (Bing *et al*., 2009) has been reported.

2.2. EFFICACY OF DIFFERENT PLANT PARTS AGAINST INSECT PESTS

Extracts from young leaves of *Parthenium hysterophorus* Linn. showed maximum antifeedant action against *S. litura* (Gajendran and Gopalan, 1982). Maximum mortality with the extracts of seeds of *Solanum dulcamara* as compared to other plant parts was reported by Chandel *et al*. (1984). According to Verma and Srivastava (1988), alcohol extract (4%) of fruits of *Solanum indicum* was the most effective against aphid *Macrosiphum rosae*. Works done by Morallo-Rejesus *et al*. (1990) found that fruits of *Capsicum frutescens* showed contact toxicity and also had an effect on the development of *C. chinensis*.

But, in *Nigella sativa* L. seed powder resulted in highest deformed adults of *Rhizopertha dominica* F. In neem also seeds caused highest ovicidal and oviposition deterrent action against *Earias vitella* F. (Gajmer *et al*., 2002). Seed kernels of custard apple were observed to be effective against *Chilo partellus* Swin.; brown plant hopper, *Nilaparvata lugens* Stal.; *S. litura*; *Spodoptera frugiperda* J.E.Smith; *Helicoverpa armigera* Hubner.; hairy caterpillar *Spilosoma obliqua* Wk., brinjal spotted leaf beetle, *H. vigintioctopunctata*., *Dysdercus koenigii* F., semi- looper *Achoea janata* Linn. and aphids (Saito *et al*., 1989; Rao *et al*., 1990; Bhagawan *et al*., 1992; Ghatak and Bhusan 1995; Hiremath *et al*., 1997; Bhatnagar and Sharma 1997; Mathew *et al*., 1999; Raman *et al*., 2000; Sonkamble *et al*., 2000; Bhuiyan *et al*., 2001).

2.3. EFFICACY OF SOLVENT EXTRACTS OF BOTANICALS AGAINST INSECT PESTS

Tan and Sudderuddin, (1978) observed that aqueous or alcoholic neem seed kernel extract (NSKE) at 5000 and 10000 ppm per liter of water showed moderate reduction in the rate of emergence of *P. xylostella* eggs. Laboratory and field experiments conducted in China to determine the effectiveness of extracts of *Melia toosendan* Sieb, *Melia azaderach* L. and *A. indica*. against arthropod pests of agricultural crops, petroleum ether extracts of the seed kernels of *M .toosendan* and *M. azaderach* at six per cent showed strong ovipositon deterring effects on *Orseolia oryzae* Wood Mason(Chiu, 1985). Field trials conducted by Verkerk and

Wright (1993) showed that water extract of neem seed kernels was more effective than its methanol neem seed kernel extract (NSKE) against *P. xylostella*. Nair, (1996) studied the ovicidal action of *Acorus calamus* L. extracts on melon fly *Bactrocera cucurbitae* Coquillett. by exposing the eggs to *A. calamus* extracts. Jaglan *et al*. (1997) evaluated the effect of *A. indica* extracts against *H. armigera* and reported that Chloroform : methanol (9:1) extracts of neem seed kernels and leaves showed better insecticidal properties than methanol extracts. Aqueous extracts of *Calotropis procera* Ait. and *Datura stromonium* L. displayed 90 per cent feeding protection against *H. armigera* (Dodia *et al*., 1998). Janarthan *et al*. (1999) reported that petroleum ether extracts of *P. hysterophorus* at 0.2 and 0.5 per cent caused 100 per cent mortality of *H. armigera* and 0.1 per cent methanol extract showed highest ovicidal action.

Nair and Thomas (2001) reported that both aqueous and solvent extracts of *A. calamus* showed ovipositonal deterrency against the melon fly *B.cucurbitae* Methanol extract of *Momordica charantia* L. leaves showed strong oviposition deterrent activity against *Liriomyza trifolii* Burgess. (Mekuria *et al*., 2005).

When different solvent extracts of seed kernels of *Annona squamosa* L. were tested against red flour beetle (*Tribolium castaneum* Herbst.) highest mortality was observed in petroleum spirit extract where as ethyl acetate and acetone extracts were moderately toxic and methanol extract was the least toxic (Khalequzzaman and Sultana, 2006). Organic extracts from the leaves of *Vitex mollis* when assessed for their toxic effect on neonate larvae of *S. frugiperda,* chloroform – methanol 1:1 extract was found to be most active and showed greater insecticidal activity (Lopez *et al*., 2007). Antifeedant and larvicidal activity of the acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *Ocimum canum, O. sanctum* and *Rhinacanthus nasutus* were studied against fourth instar larvae of *S. litura*. The highest larval mortality was found in methanol extract of *O. canum, R. nasutus* and acetone extract of *O. sanctum* against *S. litura* (Kamaraj *et al*., 2008).

2.4. MODE OF ACTION OF BOTANICALS

2.4.1. Ovipostional deterrency

Aqueous suspension of the seed kernel of neem (2%) had effective oviposition deterrent action against *S. litura* (Joshi and Sitaramaiah, 1979). Fagoonee (1984) investigated the behavioral response of *Crocidolomia binotalis* Z. to neem extracts and found that, crude extracts of dried neem leaves repelled females of the insect even from a distance of about 25cm preventing oviposition. Subadrabai and Kandaswamy (1985) recorded complete suppression of egg laying of *S. litura* and *H. vigintioctopunctata* when treated with acetone extracts of *V. negundo*. According to Schmutterer (1990), neem products repelled females of *C. binotalis, H. armigera, S. frugiperda, Lucilla serricator* (Mg.) and *Callosobruchus* spp. from oviposition on the host plants.

Gallic acid, a common allelochemical in woody plants had significant ovipositonal deterrency against *S. litura* (Mukherjee and Sharma, 1993). Crude extracts of *A. paniculata* prevented egg laying of *P. xylostella* as reported by Hermawan *et al*. (1994). Experiment conducted by Naumann and Isman (1995), reported that one per cent crude oil emulsion of neem seed significantly reduced the proportion of eggs laid by *S. litura* on treated plants. According to Patel and Patel (1998), leaf extracts of *Ailanthus excelsa* Roxb. at two and four per cent deterred oviposition of *S. litura* on the treated surface.

In laboratory studies, extracts of *A. paniculata* acted as an oviposition deterrent to *Callosobruchus chinensis* L. (Hermawan *et al*., 1993). The compound identified from *A. paniculata* was 14-de-oxy andrographolide and at 1000 ppm reduced the number of eggs laid by *P. xylostella* by about 50 per cent (Hermawan *et al*., 1998). Ethyl acetate fraction of *A. paniculata* possessed highest ovipositional deterrency against *Spilarctia obliqua* Walker. (Tripathi *et al*., 1999).

According to Tare (2000), medicinal plant oils (*A. calamus, A. indica, Eucalyptus* sp., *Pongamia glabra* Vent. Sans. *etc*) showed cent per cent ovipositonal deterrency against *P. operculella, S. litura and A. janata*.

An experiment conducted by Raja *et al*. (2003a) reported that the hexane extract of *A. calamus* roots and the ethyl acetate extracts of *Artemisia nilagirica* Clarke. showed significant ovipostion deterrent activity against *S. litura*. According to Bajpai and Sehgal (2003) among different solvent extracts of neem seed kernels, methanol extract at 0.1, 0.15 and 0.2 per cent, chloroform extract at 0.15 and 0.2 per cent and butanol extract at 0.2 per cent strongly inhibited the ovipositon of *H. armigera* moths. Experiments conducted by Singh *et al*. (2006) proved the oviposition deterrent activity of methanol extract of ginger against *H. armigera*. Cotton leaves when treated with *Pedalium murex* L. root ethanol extracts reduced the ovipositional duration and the number of eggs laid in case of *Dysdercus cingulatus* F. (Sahayaraj *et al*., 2006). In experiments when conducted to study the effect of different plant extracts (nishinda – *V. negundo*, eucalyptus – *Eucalyptus globules* Labill., bankalmi – *Ipomoea sepiaria* K.) against *C. maculatus*, the lowest number of eggs were laid in the food treated with nishinda, followed by eucalyptus and bankalmi at an extract concentration of 2% (Rahman and Talukder, 2006).

2.4.2. Ovicidal action

An experiment conducted by Yadav in 1985 showed that neem oil had a strong ovicidal action against three species of *Callosobruchus*. Bhathal *et al*. (1991) observed that the *Ageratum conyzoides* extracts showed more pronounced ovicidal action against *D. koenigii*. Volatile oils from *V. negundo* caused upto 83 per cent mortality of *P. xylostella* eggs (Dayrit *et al*., 1995). Suryakala *et al*. (1995) reported that the root extract of *Vetiveria zizanoides* (Linn.) inhibited 75 to 90 per cent and 50 to 80 per cent egg hatching of *S. litura* and *D. koenigii* respectively. Methanol extract of *A. squamosa* had ovicidal action on the eggs of *S. obliqua* at two per cent concentration (Khatak and Bhusan,1995). They had also observed that methanol and petroleum ether extracts at one per cent inhibited *S. obliqua* eggs from hatching. Ovicidal activity of oils of *A. indica, Milletiaie ferruginea* and *Chrysanthemum cinererifolium* against *Callosobruchus chinensis* was reported by Mulatu and Gebremedhin (2000). Studies conducted by Pandey

and Khan (2000), revealed the ovicidal effect of *Clerodendron siphonanthus* leaf extracts against *C. chinensis*. Tripathi *et al*. (2003a) have reported the ovicidal action of leaf essential oil of *Aegle marmelos* against *S. obliqua* when applied @ 250mg oil/50 eggs. Hexane and methanol extract of *A. calamus* roots and ethyl acetate extracts of *Wedelia calendulacea* showed significant ovicidal activity against *S. litura* (Raja *et al*., 2003b). Studies conducted by Pavunraj *et al*. (2006) indicated that hexane extract of *Excoecaria agallocha* L. had ovicidal activity against *S. litura*. Treatment with *Pedalium murex* L. root extracts resulted in reduced hatchability in case of *D. cingulatus* (Sahayaraj *et al*., 2006). Parthenin, a sesquiterpene lactone, obtained from wild fever dew, *Parthenium histerophorus* was converted into different derivatives by chemical and photochemical transformations and tested for their ovicidal activity against red cotton bug. The derivative, pyrazoline of parthenin had maximum ovicidal action for both 0-24 and 24-48 h old eggs (Sharma *et al*., 2006). According to Singh *et al*. (2006) minimum hatchability of eggs of *H. armigera* (48%) was recorded in two per cent hexane fraction of ginger and it was statistically at par with 10 per cent methanol extract

2.4.3. Growth and developmental effect

The effect of an extract of Eucalyptus leaves on *S. litura* was studied by Chocklingam *et al*. (1986). In this study, it was found that the duration of the fifth larval instar was prolonged by two days when fed on leaves of castor treated with the extract at 300 and 400 ppm.

Azadirachtin when added to semi-synthetic diet, inhibited neonate larval growth in a dose-dependent fashion in *S. litura* and *Trichoplusia ni* (Isman, 1995). Indian laurel (*Laurelia* sp.) and *A. indica* extracts prolonged larval duration of *S. litura* (Behera and Satapathy, 1996). Reduced growth rate was observed in azadirachtin treated *S. litura* larvae and the main reason for reduced growth rate was due to the drastic reduction in the activity of gut trypsin (Koul *et al*., 1995). Gujar (1997) found that the sub lethal dose of azadirachtin against *H. armigera* led to drastic growth inhibition for a considerable period of time. Custard apple

extract showed highest activity by reducing the larval growth and total development, prolonged larval duration, lowered pupal weights, resulting in the formation of deformed individuals (Vyas *et al*., 1999). Feeding trials with azadirachtin on *S. littoralis* showed that a two-day feeding period was enough to promote prolongation of the larval instars and reduction in mean relative growth rate (MRGR) (Martinez *et al*., 2001). Studies conducted by Koul *et al*.(2004a) reported that nimbocinol exhibited growth inhibitory activity in artificial diet bioassays with 82.4 and 92.2 mg kg $^{-1}$ concentrations inhibiting growth by 50 per cent when tested against *H. armigera* and *S. litura*. Larval duration of elm leaf beetle (*Xanthogalurica luteola*) was increased when treated with the methanol extract of the plant, *Artemicia annua* L. (Shekari *et al*., 2008). Essential oil of *V. trifolia* and *V. aganus-castus* were evaluated against fifth instar larvae of *S. obliqua* and found that the treatment caused extended larval and pupal period (Tandon *et al*., 2008). Nathan *et al*. (2009) reported that larval duration of rice leaf folder was prolonged with a reduction in weight on treatment with pure triterpenes isolated from *Dysoxylum malabaricum* Bedd.

2.4.4. Antifeedant and repellant action

Many plant compounds appear to protect a plant by repelling an insect or deterring feeding. Many compounds have been characterized as acting as antifeeding deterrents, deterring feeding through behavioural modification following detection by chemosensilla. The antifeedant would inhibit feeding, thus protecting the crop without killing the insect directly, although the insect may die indirectly due to starvation.

Mane (1968) reported the repellent action of neem seed suspension against *Euproctis lunata* Walk., *Prodenia litura* F., *Utetheisa pulchella*, *Acrida exaltata* L. and *Aulacorphora foveicollis* L. Studies conducted by Patel *et al*. (1968) revealed the deterrency of neem seed paste suspension against the hairy caterpillar *Amsacta moorie* Butl. Laboratory tests conducted by Joshi and Ramaprasad (1975) showed that a three per cent suspension of seed kernels of neem (*A. indica*) was effective as an antifeedant against all the five instars of *S.*

litura. In a laboratory study in Japan, chlordimeform and four natural antifeedants were assayed by the leaf disc method for antifeedant activity in starved fifth instar larvae of *S. litura*. In this study, the natural antifeedants clerodin and clerodendrin B recorded the highest antifeedancy (Antonious and Saito, 1981). According to Koul (1983) two limonoids isolated from *Cedrella toona* and grape fruit when sprayed on castor leaves were found to have feeding deterrence to *S. litura*. In choice and no choice tests conducted in China with fifth instar larvae of *S. litura* using the leaf disc method, the methanol extracts of the seed kernels of *Melia azadirach* and *M. toosendan* showed antifeedant activity.

Gunasekaran and Chelliah (1985a) reported that the acetone extract of *A. paniculata* had higher antifeedant activity against third instar larvae of *S. litura* than the aqueous extract. Among the seven species of plants tested against third instar larvae of *S. litura* for antifeedant activity, seed extract of mahogany, a whole plant extract of *A. paniculata* and karanj oil had the highest activity (Rajasekaran and Kumaraswami, 1985). Experiments conducted by Koul (1987) reported that at concentrations of 0.5 per cent and one per cent, calamus oil was effective in inducing a significant reduction in feeding in early third instar larvae of *S. litura*.

The antifeedant activity of neem seed extract formulations were tested against fourth instar grubs and adults of *H. vigintioctopunctata* in the laboratory. Neem-75 at 1000ppm was the best antifeedant for larvae, followed by NK.100 and Nemidin. NK100 had the greatest antifeedant activity against adults. (Jayarajan and Babu, 1990).

In a leaf disc choice bioassay, aristolochic acid 2 and 4 showed the strongest antifeedant activity against the larvae of *S. litura* when compared to phenanthrene, 1-3 bezo dioxide and reduced aristolochic acid derivatives (Lajide *et al*., 1993). Different plant extracts were tested to evaluate their antifeedant property against *S. litura*. Studies revealed that treatment with *V. negundo* extracts against *S. litura* resulted in maximum leaf protection when compared to other plant extracts (Sahayaraj, 1998).

Antifeedancy of leaf extracts of *T. nerifolia* to *H. vigintioctopunctat*a and *S. litura* (Sardamma, 1989) and to *Athalia proxima* (Klug) (Pandey *et al*., 1976) were reported. Studies conducted by Murugan *et al*. (1998) to evaluate certain tropical plant extracts for their antifeedant action against *S. litura* showed that the plants *Strychnos nuxvomica*, *Nicotiana tabacum* and *Capsicun anum* recorded the highest antifeedancy of 100 per cent followed by *Calotropis gigantea* and *Datura metel* (90 and 81 per cent respectively). According to Babu *et al*. (1998) the crude oils from seeds of *Annona sqamosa* and *A. reticulata* at 2.5 and 5 per cent concentrations significantly reduced leaf damage caused by *S. litura*. Morimoto *et al*. (1999) reported the antifeedant activity of stems of *Cyperus* spp. Considerable feeding inhibition (80.21%) was recorded for third instar larvae of *S. obliqua* treated with 0.4 per cent *Artemisia nilagirica* (Clarke) Pamp. (Chowdhury *et al*., 2000). Wheeler and Isman (2001) reported the antifeedant activity of a crude methanol extract of *Trichilia americana* against *S. litura*. The antifeedant activity of *Tephrosia vogelii* (Hook) against *S. litura* was reported by Zhang-Yi *et al*. (2004).

Twenty-nine solvent extracts from 20 Sri Lankan plants were examined for their antifeedant activity against the fourth instar larvae of Mexican bean beetle, *E. varivestis.* Extracts of *Sarcocolla brevifolia, Strychnos nux- vomica, Syzygium caryopyllatum* and *Feronia limonia* showed strong antifeedant activity (Jayasinghe *et al.,* 2003). Treatment with neem limonoids 3-O- acetyl salannol, salannol and salannin deterred feeding by 50 per cent in *S. litura* larvae (Koul *et al*., 2004b).

Antifeedant and growth inhibitory effects of crude plants extracts (*Melia volkensii* and *Organum vulgare*) and pure allelochemicals (digitoxin, cymarin, xanthotoxin, toosendanin, thymol and trans- anethle) were investigated against cabbage looper (*T. ni*), diamond back moth (*P. xylostella*) and Mexican bean beetle (*E. varivestis*) using leaf disc choice bioassay. *M. volkensii* was the most potent growth inhibitor for *T. ni.* and feeding deferent for third instar *P. xylosella* and adults of *E. varivestis.* (Akthar and Isman, 2004).

Among the different medicinal plants, the leaf essential oils of *V. negundo* caused 100 per cent deterrence of feeding against *S. litura* third instar larvae (Sharma *et al*., 2001). In an experiment conducted by Sabitharani and Murty (2003), rhizome extracts of *A. calamus* at one per cent concentration resulted in 100 per cent feeding deterrence against the third instar larvae of *S. litura*. Studies conducted by Raja *et al*. (2003b) indicated maximum antifeedant activity in *S. litura* when treated with ethyl acetate extract of *Hyptis suaveolens*. According to Mehta *et al*. (2005) aqueous extracts of *Lantana camera* L. resulted in maximum antifeedancy against larvae of *P. xylostella* than *Murraya koenigii* L., *M. azadiracta* and *Rumex nepalensis* Spring. Antifeedant activity of 73.08 per cent was observed in leaf extract of *E. agallocha* against *S. litura* (Pavunraj *et al.,* 2006). An experiment conducted by Ignacimuthu *et al.* (2006) revealed antifeedant activity of methanol extract of *Sphaeranthus indicus* against fourth instar larvae of *S. litura*. Among the compounds isolated from this fraction, 7-hydroxy frullanoide had highest antifeedant activity at 1000ppm. Rahman and Talukder (2006) evaluated the effects of different plant extracts on pulse beetles by comparing the total number of eggs laid, egg hatching per centage and inhibition rates in the treated and control black gram seeds. The lowest hatching rate was observed in the black gram seeds treated with the plant extracts bankalmi (*I. sepiaria*) and nishinda (*V. negundo*) at three per cent concentration.

While detecting the bioactivity of major sesquiterpenes of *Chloroxylon*, it was observed that the components geijerene and pregeijerene displayed maximum feeding deterrence to *H. armigera* (Ravikiran *et al*., 2007).

2.4.5. Nutritional effect

Toxic effects of chemicals occur following ingestion of the compound. A compound may show acute toxicity, resulting in the rapid death of the insect or chronic toxicity, where the effect is less dramatic, often resulting in symptoms similar to reduced ingestion.

Food consumption studies conducted by Chockalingam *et al*. (1986) showed that the duration of fifth larval instar of *S. litura* was prolonged by 2 days when

fed on castor leaves treated with eucalyptus extracts at 300 and 400ppm. Food consumption, assimilation and production were inversely proportional to the concentration of extract tested. The limonoids might act primarily as antifeedants. They were known to be very bitter substances and many experiments have shown that observed decreases in growth is due to a decrease in food intake (Koul, 1983; Vanucci *et al*., 1992; Mendal *et al*., 1993). Azadirachtin (Az) was reported to be reducing the food intake and approximate digestibility in *S. litura* (Rao *et al*., 1993). The consumption index (CI) of *Taragama siva* Lefbvre is considerably reduced by methanol extract of neem seed kernel powder (NSKP) (Sundararaj *et al*., 1995). Dietary concentrations of azadirachtin significantly lowered the efficiencies of conversion of both ingested (ECI) and digested (ECD) food, but failed to lower the approximate digestibility (AD) of *S. litura* larvae (Mukherjee and Sharma, 1996). Effect of certain plant extracts on the consumption of food by *S. litura* was evaluated (Chitra and Rao, 1996). The order of efficacy of plant extracts with respect to reducing the food consumption was NSK (5%) and *N. odoram* (5%) and *A. mexicana* (1%). Studies conducted by Sahayaraj (1998) revealed that among the different plant extracts tested against *S. litura* larvae, lowest food consumption, Approximate digestibility (AD) and ECD were observed in insects treated with *V. negundo* extracts.

Haque *et al*. (1996) reported that neem oil significantly reduced the food consumption of the third instar larvae and adults of epilachna beetle. According to Anam *et al*. (2006) neem oil significantly reduces the rate of food consumption of epilachna beetle (*Epilachna dodecastigma* Wied.) in second instar grubs.

The biological activity of a crude methanol extract of *Trichilia americana* was assessed using the Asian army worm, *S. litura*. In nutritional assays, the crude extract reduced growth, consumption and the utilization of ingested and digested food in a dose dependent manner (Wheeler and Isman, 2001). The azadirachtin preparation, Neemazal, was assessed against the Egyptian cotton leaf worm, *Spodoptera littoralis* Boisd. to evaluate its possible action on food metabolism. Results revealed that all the nutritional parameters like Relative Consumption Rate (RCR), Relative Growth Rate (RGR), Assimilation Rate (AR), Efficiency of

Conversion of Ingested Food (ECI) and Efficiency of Conversion of Digested Food (ECD) showed a drastic decline (Ahmed *et al*., 2003).

Among the different compounds isolated from *A. indica*, nimbocinol and azadiradione reduced ECI in feeding experiments indicating toxicity rather than antifeedant effects (Koul *et al*., 2004b). Histological changes in the alimentary canal of *S. litura* treated with botanicals and conventional insecticides were studied by Vijayaraghavan *et al*. (2003). Results revealed that epithelial cells were heavily damaged in annona seed extract treated larvae than the neem treated larvae. The bioefficacy of aglaroxin A from *Aglaia elaeagnoidea* was assessed using the gram pod borer, *Helicoverpa armigera* (Hubner) and Asian army worm *S. litura*. The growth efficiency of the larvae fed on treated diet was significantly less than that of control larvae (Koul *et al*., 2005). Studies on the insecticidal properties of ginger, *Zingiber officinale* roots against *Earias vittella* were conducted in CCS Haryana Agricultural University. In consumption and utilization studies, consumption index ranged from 3.40 to 2 per cent in benzene extract to 8.64 to 2.5 per cent in methanol extract. ECI and ECD was lowest in 1.5 and 2 per cent acetone fractions (Singh *et al*., 2005). According to Nathan *et al*. (2005), when *S. litura* larvae were chronically exposed to neem limonoids, 59–89 per cent reduction in weight was observed along with a strong inhibition of the gut enzymes, acid phosphatases (ACP), alkaline phosphatases (ALP) and adenosine triphosphatases (ATP ase). Experiments were conducted to evaluate the efficacy of *A. annua* extracts against *X. luteola* adults and results shown that the nutritional indices like Consumption Rate (CR), Approximate Digestibility (AD), Conusmption Index (CI), ECI and ECD were significantly reduced in treated insects (Shekari *et al*., 2008).

Liu *et al*. (2009) report that when the botanical, fraxinellone was incorporated into artificial diets it significantly reduced the relative growth rate, food consumption rate as well as the efficiency of conversion of ingested food into biomass of *Heliothes virescens*. They also report that after treatment the larval midguts of *H. virescens* possessed significantly lower activities of ά amylase and non specific proteases.

2.4.6. Insect Growth Regulating Activity

Growth regulating activity of neem products have been reported on many pest species *viz*. *C. medinalis* and *N. lugens, Mythimna separata* in rice (Saxena *et al*., 1980); *Heliothis zea, S. frugiperda, Pectinophora gossypiella* (Sau.) and *H. virescens* in cotton (Kubo and Klocke, 1982) and *Spodoptera exigua* (Hub.) and *T. ni* (Prabhakar *et al*., 1986) in vegetables. Insect growth regulating factors as antijuvenile hormonal agents have been reported from the extracts of the common goat weed *Ageratum conyzoides* Linn. (Fagoonee and Umrit, 1980). Petroleum ether extract of *Tribulus terrestris* Linn. had juvenile hormonal effects against *S. litura* and *H. armigera* (Gunasekaran and Chelliah, 1985b). An experiment was conducted by Rao and Subrahmanyam (1987) to find out the effect of azadirachtin on *S. litura*. The female adults of *S. litura* which emerged from treated larvae had prolapsed anal brushes. The bioactive constituents from the leaves of *Machilus japonica viz*. neolignans, licasin A and machilusin exhibited growth inhibition activity against *S. litura* larvae (Gonzalez *et al*., 1994). Moult inhibitory action of azadirachtin on *S. litura* larva was reported by Koul *et al*. (1995). Laboratory experiments conducted by Govindachari *et al.* (1996) showed that salanin, one of the active principle in neem, delayed moulting, caused larval and pupal mortalities and decreased pupal weights in *S. litura* and *Pericalia ricini*. Salanin also caused moult delays and nymphal mortalities in *Oxya fuscovittata*.

Among the different indigenous plant extracts tested, sweet flag induced the highest percentage of abnormalities against *S. litura* (Behera and Satapathy, 1997). Tripathi *et al.* (1999) reported that the methanol fraction of *A. paniculata* extracts had the highest growth inhibitory activity on larval and adult stages of *S. obliqua.* At higher concentrations, ether extracts of *A. squamosa* treated *S. litura* larvae showed larval-pupal and pupal adult intermediary forms (Mani and Prabhu, 1999).

A. indica, *Chromolaena odorata*, *Clerodendron infortunatum, Lantana camara*, *Nerium oleander* and *Ocimum sanctum* crude extracts treated *D. cingulatus*, nymphs developed into malformed adults (Saradamma, 1989). Plant

extracts of *A. indica, C. sinensis, V. negundo* and *Zingiber officinale* applied on *S. litura* produced deformed pupae as well as adult stages (Sahayaraj, 1998).

According to Parvathi *et al.* (1999) topical application of 100-300 µg of methanol extracts of *Glyricidium sepicum* Jacq. at different stages of *D. koenigii, A. janata* and *S. litura* resulted in larval-pupal intermediates and deformed adults in a dose dependent manner. Sahayaraj and Paulraj (2000) reported that the crude extracts of *Tridax procumbens* when applied on fourth instar *S. litura* larvae developed into deformed pupae and adults.

Rhizome extract of *A. calamus* is having insect growth regulatory activity as well as juvenile hormonal effects (Deshmukh and Renapurkar, 1986). Saradamma *et al.* (1993) reported the juvenomimetic activity of *V. negundo* on the penultimate instars of *D. cingulatus*. Sahayaraj (1998) reported the morphogenetic effects of *V. negundo* extracts on *S. litura* larvae.

When larvae of *Culex fatigans,* were treated with 0.50 per cent *V. negundo* extracts, 38 per cent of the larvae emerged as pupae, but failed to reach the adult stage. At 0.25 per cent concentration, 52 per cent of the larvae emerged as pupae but also failed to reach the adult stage (Shah and Maheshwari, 2002). Solvent as well as aqueous extracts of *V. negundo* had higher rate of pupal and adult malformations when applied on *S. litura* (Suresh, 2002).

Kalavathi *et al.* (1991) reported that the acetone extract of *V. negundo* could inhibit the emergence of *Epilachna septima* adults, if pupae were treated with the same. Topical application of ethanol extract of leaves of *A. conyzoides* reduced adult emergence up to 80 per cent at 2000 µg when applied to *S. litura* larvae (Sharda *et al*., 2000).

Acetone extract of *Aristolochia bracteata* Retz. at 5, 10, 15 and 20 per cent concentrations were tested against the tobacco cut worm *S. litura. A. bracteata* extract caused adult and pupal mortality, and arrested adult emergence (Senthilkumar, 2001). An experiment conducted by Sharma *et al*. (2003) showed that the alcohol extract of *Tinospora cordifolia* when tested against *S. litura* was more effective than neem extract both in terms of mortality and IGR activity.

Inhibition of larval growth was observed in crude ethaol seed extracts of *A. squamosa* and *A. moricata* (Leatemia and Isman, 2004).

Maximum IGR activity (84.21%) was observed from the topical application of acetone extract of *Catharanthus roseus* leaves against the larvae of *S. litura* (Singh *et al*., 2005). Growth inhibition and larval duration increased with increasing rates of azadirachtin (Zhang-Yi *et al*., 2004). Aglaroxin A, the compound isolated from the plant *Aglaia elaeagnoidea* exhibited a strong growth inhibition of 95 per cent at 2.36ppm and 2.41ppm in *H. armigera* and *S. litura* respectively (Koul *et al*., 2005). According to Tandon *et al*. (2008), when essential oils of *V. trifolia* and *V. aganus-castus* were tested against fifth instar larvae of *S. obliqua*, adult deformities and reduction in the emergence of adults were observed.

2.4.7. Longevity

Treatment with vegetable oils (corn, ground nut, sunflower and sesamum) significantly reduced the longevity of adults of *Callosobruchus maculatus* and *C. chinensis*. Longevity of *C. rhodsianus* was significantly reduced by treatment with corn and sunflower oil (Rajapakse and van – Embden, 1997).

2.4.8. Fecundity

Kumar and Babu (1998) reported that Azal – T/S (1% azadirachtin) and neem azal F (5% azadirachtin) have adverse effects on the fecundity of *H. vigintioctopunctata*. Treatment with chloroform, methanol and petroleum ether extracts of *Adathoda vasica* Nees. lowered fecundity of *Helopeltis theivora* Waterhouse (Deka *et al*., 1999). Reduction in fecundity and egg fertility of *S. obliqua* were observed on treatment with essential oils of *V. trifolia* and *V. agnus-castus* (Tandon *et al*., 2008). Methanol extract of *A. annua* was investigated for its toxic effect on elm leaf beetle, *Xanthogaleruca luteola*. Adult fecundity and fertility was significantly reduced with abnormal ovaries in the treated insects when compared to control (Shekari *et al*., 2008).

2.4.9. Insecticidal action

Chopra *et al*. (1949) listed about 700 spp. of plants having poisonous effects on man, livestock and insects, out of which, 74 plants showed insecticidal and insect repellent properties.

Neem leaf extracts at two and five per cent levels killed the larvae of *Epilachna varivestis* (Mul.) in beans and *P. xylostella* in cabbage respectively (Steets, 1975). Insecticidal effect of powdered rhizomes of sweet flag, *A. calamus* was recognized against mosquitoes, houseflies, pulse beetles, bird lice and bed bugs (Subramanian, 1942).

Insecticidal properties of essential oil of *A. calamus* were reported against various storage pests (Koul, 1967; Abraham *et al*., 1972; Agarwal *et al*., 1973 ; Yadav, 1974). Ether extract of *A. calamus* was toxic to *A. proxima* (Pandey *et al*., 1976 and Sudhakar *et al*., 1978). In another experiment Pandey *et al*. (1982) reported that two per cent extracts of the rhizomes killed fifth instar larvae of potato tuber moth *Phthorimaea operculella*. Kalyanasundaram and Babu (1982) reported that petroleum ether extract of leaves of *V. negundo* was effective against mosquitos. Acetone extracts of *V. negundo* caused cent per cent mortality of *S. litura* and *H. vigintioctopunctata* (Saradamma, 1989; Moore *et al*., 1989). Kalavathy *et al.* (1991) reported the insecticidal activity of acetone extracts of *V. negundo* against *Earias vitella*. Laboratory and nursery trials in Andhra Pradesh, India during 1990 - 1991 showed that crude methanol and dichlorothane extracts of deoiled neem cake could be used effectively for protecting tobacco seedlings from the attack of *S. litura* (Murthy *et al*., 1992).

Murugan *et al.* (1999) found that the seed extract of *Pongamia glabra* was highly toxic to *S. litura*. Leaf extract of ginger caused 70 per cent larval mortality in *S. litura*, 96 hours after treatment (Sahayaraj and Paulraj, 1998). Sitaphal seed extract at 1.5 per cent concentration recorded the highest mortality in *H. armigera* (Sonkamble *et al*., 2000). Water extract of *Lantana camera* Linn. caused more than 30 per cent mortality against *S. litura* and *Liriomyza erysimi* (Desai and Desai, 2000).

Shanmugapriyan and Kingsly (2001) have reported that neem oil at 0.25 and 0.5 per cent caused high mortality of second and third instars (95.23%) of *H. vigintioctopunctata*. Twenty one essential oils were tested for insecticidal activity to third instar larvae of the tobacco cut worm, *S. litura.* Oils of *Satorja hortensis, Thymus serpyllum* and *Origanum creticum* produced more than 90 per cent larval mortality in 24 hrs at a dose of 100 µg/larva (Isman 2000).

Neem Azal-7/s (neem seed Kernel extract containing 1% azadirachtin), Neudosan (Potassium soap + rape oil), Spruzit flussiy 9(natural pyrethrum+ pyrethrin + piperonyle butoxide) at different concentrations were evaluated against the second and fourth instar larvae of *Epiricania chrysomelina* F. under laboratory conditions. In case of acute effects, the highest mortality was recorded when larvae were offered squash leaves treated with the highest concentration (2%) of Neem Azal –T/S (Moniem *et al.*, 2004). Among different neem limonoids tested against *S. litura* Azadirachtin was the most potent one causing 100 per cent larval mortality (Nathan *et al*., 2005).

Five different plant materials were evaluated as protectants for milled rice against lesser grain borer (*Rhizopertha dominica*) at 0.25, 0.5 and 1.0 per cent. The rhizome of sweet flag (powder) was highly toxic to the adult insects at all concentrations (Harish *et al*., 2003). Among the plant products, *A. calamus* recorded the lowest pest population. Jiyavorranant *et al.* (2003) have reported that the *A. calamus* extract gave 63.3 per cent mortality of *P. xylostella* larvae within 48 hours. Hexane, ethyl acetate and methanol extract of *Artemisia nilagirica* and hexane extract of *A. calamus* roots showed significant insecticidal activity against *S. litura* (Raja *et al*., 2003a).

Plant derived compounds, *i.e*. carvone, cineole, salicylic acid, camphene, menthol and phellandrene, each at 0.001, 0.01 and 0.1 per cent concentrations were screened against third instar larvae of *S. litura* under laboratory conditions. Of the six compounds, carvone, cineole and salicylic acid possessed insecticidal property (Gayathri *et al*., 2003). According to Perumal *et al.* (2004) the petroleum ether extract of *V. negundo, Argemone mexicana, Datura metel* and *A. squamosa* showed significant larvicidal activities against *S. litura*. Studies

conducted by Rathi and Gopalakrishnan (2005) on the insecticidal properties of the plant *Synedrella nodiflora* revealed that the methanol extract (0.08%) was the most toxic one resulting in cent per cent mortality to *S. litura* larvae. Insecticidal action of different plant extracts against *H. armigera* was evaluated by Ramya *et al*. 2008. According to them, the leaf aqueous extracts of three plants namely *A. paniculata*, *C. roseus* and *Datura metel* exhibited high rate of mortality (72.8, 67.8 and 62.2 per cent respectively) against the larvae of *H. armigera*. Biological efficacy of different solvent extracts of leaves of *Atlantia monophylla* were studied against third instar larvae of *H. armigera*. Hexane extract was subjected to fractionantion and the ninth fraction at 1000 ppm resulted in 100 per cent pupal mortality (Baskar *et al*., 2009)

2.5. EFFECT OF ACTIVE INGREDIENTS OF BOTANICALS ON INSECTS

More than 35 constituents of neem, classified under terpenoids and flavanoids, have been isolated from seeds and tested against different insect pests. Azadirachtin, the main bioactive component of neem has been identified as a potent antifeedant for different insect pests *viz*., *Locusta migratoria* (Butterworth and Morgan, 1971), *Oncopeltus fasciatus* Dallas and *S. frugiperda* (Redfern *et al*., 1981), *Diaphania indica* (Chitra and Kandaswamy, 1988), *S. littoralis, S. frugiperda, Heliothis virescens* Fabr. and *H. armigera* (Blaney *et al*., 1990).

Besides azadirachtin, meliantriol (Lavie *et al*., 1967) also deterred insects from feeding. Salannin, a substance structurally related to azadirachtin was reported to be effective at 0.005 per cent against *S. littoralis* and 0.01 per cent against *Earias insulana* Boisd (Meisner *et al*., 1978). Nine compounds from the seeds of *A. indica* namely, imbolinin-B, nimbolidim A and B, Ohchinolid A and B, diacetyl vilasinin, 3 deacetyl salannin, salannol and nimbondiol compounds exhibited antifeedant activity against *Epilachna varivestis* Muls.(Kraus *et al*., 1980).

Yojima *et al.* (1977) isolated and identified six feeding deterrent principles namely isopimpenellin, beryapectin, xantheloxin, kokusogin, evoxine and

japonine from the leaves of *Orirea japonica* Thumb. These were found effective against *S. litura*. The alkaloids like vasicine, vasicinol, deoxyvasicine, vasicinone and deoxyvascinone isolated from *Adathoda vasica* deterred the feeding of Epilachna and Aulacophora beetles at 0.05 to 0.1 per cent concentrations on vegetable crops (Saxena *et al*., 1986).

'Neriifolin' isolated from *Thevetia thevetionides* acted as stomach poison against *O. nubilalis* (Mc-Laughlin *et al*., 1980). Srimannarayana and Rao (1985) found that the Karanjin from *Ponagaima glabra*, 'maxima substance' from *Tephrosia purpurea* Pers. and lonchocarpic acid from *Derris scadens* (Roxb.) were effective against *S. litura*. In an experiment conducted by Rani and Jamil (1989) petroleum ether extracts of water hyacinth when mixed with larval diet of *Tribolium castaneum* and *Corcyra cephalonica* caused mortality in the fourth larval instar. Water extract of leaves of *N. oleander, A. indica, T. neriifolia* and
C. infortunatum produced nymphal mortality in *D. cingulatus* (Saradamma, 1989). Jacobson (1990) reported about the biologically active compounds like 'annonin, annonacin and annonidines' from custard apple. 'Plumbagin' from *Plumbago* sp. possessed antifeedant, chitin synthesis inhibition and ecdysteroid inhibition properties against a number of lepidopteran and hemipteran insect pests (Chocklingam *et al*., 1990 and Krishnayya and Rao, 1995).

An azasteroid, 25-azachotesteryl-methyl ether showed inhibitory effect on moulting and reproduction (Kaur *et al*., 1994). Li-xiaodong *et al.* (1996) reported that the main mode of action of azadirachtin (Az) was disruption of endocrine activities in insect. Crude suspension of the *A. indica* seeds had stronger combined larval mortality and antioviposition properties against *S. litura* than the pure Az (Naumann and Isman, 1995).

Antifeedant property of andrographolide against *Nephotettix cincticeps* was reported by Widiarta *et al.* (1997). The same at 1000 ppm reduced the number of eggs laid *P. xylostella* by about 50 per cent (Hermawan *et al*., 1998). Samaderin B and Indaquassin isolated from *Glochidion hochneckeri* reduced feeding by 60 per cent when treated against *S. litura*. Samaderins (quassinoids) caused an increase in the larval period (Govindachari *et al*., 2001).

The emulsifiable concentrate UDA – 24.5 based on an essential oil extract from *Chenopodium ambrosioides* at 0.5 per cent was significantly more effective in managing green house whitefly than neem oil, endosulfan and the control treatment (Chiasson *et al*., 2004). Karangin was most effective to inhibit 50 per cent normal adult emergence from the treated larvae (Lognathan *et al*., 2006).

2.6. EFFECT OF MICROBIALS ON INSECTS

2.6.1. Effect of *Bacillus thuringiensis* on *S. litura*

An experiment conducted by Sareen *et al.* (1983) in Uttar Pradesh determined the effect of different concentrations of *B. thuringiensis* var *thuringiensis* on *S. litura* in which soybean leaves were fed to the larvae. The larval mortality was found to increase with increasing doses and the first instar larvae were more susceptible than the later instars. Zaz and Kushwaha (1993) determined the pathogenicity of *B. thuringiensis* to six larval instars of *S. litura* in the laboratory at 35^0 C and 70 per cent relative humidity (RH). The mortality and deformity was highest for first instar (87.5%) and then showed a declining trend with (32.5%) to sixth instar. A decrease in mortality was found with decrease in concentration. Laboratory evaluation of different *B. thuringiensis* subspecies were conducted by Puntambekar *et al.* (1997) and found that *kurstaki* at 10^8 spores ml^{-1} caused more than 85 per cent mortality to the neonates of *S. litura*.

Laboratory experiments were conducted to test the the pathogenicity of *B. thuringiensis* subsp. *kurstaki* on different larval stages of *S. litura*. Results showed that the early instars were susceptible to *B. thuringiensis*. Higher doses were found to be effective in later instars (Gloriana *et al*., 2000). Bioefficacy of *B. thuringiensis* subsp. *kurstaki* against larvae of *S. litura* of different age groups was done by Rashmi *et al.* (2003). In the study, early instars showed higher mortality as compared to later ones. They have concluded that the older larvae were physically stronger due to manifold increase in body weight and much higher doses were needed to kill them.

2.6.2. Nuclear polyhedrosis virus (NPV)

Field-plot and laboratory tests in Madurai District in 1977 showed that foliar applications of a suspension of the NPV of *S. litura* to banana at a rate of $1.2x10^{12}$ polyhedral inclusion bodies (PIB)/ha gave effective control of larvae (Santharam *et al.*, 1978). and Ramakrishnan (1980) obtained 56.4 per cent reduction of damage by *S. litura* on cauliflower with the use of NPV at $26x10^{6}$ PIB/ml. Ramakrishnan *et al.* (1981) reported 14.48 per cent reduction in the damage of *S. litura* by using NPV of *S. litura* at $9.3x10^{9}$ PIB/ml. Jayaraj *et al.* (1981) reported 80.6 to 87.7 per cent larval mortality of *S. litura* on cotton by using NPV at 250-500 larval equivalent (LE)/ ha. An experiment conducted by Krishnaiah *et al.* (1984) showed that there was an increase in yield (16.2%) in black gram when NPV of *S. litura* was used at 1500 LE/ ha. Similarly an yield increase of 13.3 per cent was obtained in case of groundnut crop when NPV was used at 750 LE/ ha on this crop.

The potentiation of the NPV with boric and tannic acids on *S. litura* was studied by Rao *et al.* (1987). The addition of 25 per cent boric or tannic acid enhanced the mortality of *S. litura* due to NPV. Field experiments were carried out in Tamil Nadu, India in 1990 to assess the control of *S. litura* on ground nut using NPV and locally recommended chemical insecticides. Two applications of NPV and 250 LE/ ha at seven and ten days intervals were as effective as endosulfan in reducing the larval population and damage to grains in sorghum and leaves in groundnut (Dhandapani *et al*., 1993). Work done by Kulkarni and Hugar (2000) showed that mortality of *S. litura* increased with increase in NPV concentration.

2.6.3. Entomogenous fungi

2.6.3.1. Beauveria bassiana Balsamo

Ignoffo *et al.* (1982) reported that first instar larvae of *T. ni* when exposed to a leaf disc treated with viable conidia of *B. bassiana* for 48 h the LC_{20}, LC_{50}
and

LC_{80} of this fungus were 32, 139 and 590 conidia/ mm ^{2} respectively, five days after treatment.

High level of infectivity was also observed when the larvae of *Lymantria dispar* were offered fungal treated leaves of oak for 48 h (Wasti and Hartmann, 1982). Bioassay with *B. bassiana* on second, third and fourth instar larvae of *S. litura* and *H. armigera* revealed decrease in virulency of the fungus with increase in larval age. However among three isolates of this fungus, Baptla isolate showed greater virulence than Banglore and Delhi isolates towards second instar larvae of these polyphagous pests (Prasad *et al*.,1989, and 1990). Gopalakrishnan and Narayanan (1990) reported that all stages of the larvae of *H. armigera* were susceptible to *B. bassiana*, which caused 60 to 100 per cent mortality.

The fungus *B. bassiana* was highly pathogenic to pupae and adults of *Anthonomus grandis* with LD_{50} at 6 and 7 days as 1.49x10 ^{6} and 6.1x10 ^{7} conidia/ ml, respectively, for pupae and 2.88x10 ^{9} conidia/ml for adults (Wright and Chandler, 1991). Kaaya *et al.* (1993) tested three isolates of *B. bassiana* and recorded that all three isolates were pathogenic to third instar grubs of *Cosmopolites sordidus* by causing 98 to 100 per cent mortality after nine days.

The effect of *B. bassiana* on different developmental stages of *S. litura* was evaluated by Jayanthi and Padvathamma, 1996. Larval mortality decreased with decrease in microbial concentrations, higher percentage mortality was observed in the early instars compared to later instars. Hanif and Abida (1997) reported that *B. bassiana* is effective against all stages of *Epilachna dodecastigma* Wied. except eggs. In another experiment Padmaja *et al.* (1998) studied the virulence of *B. bassiana* at different developmental stages of *H. vigintioctopunctata.* Larval stages were more susceptible as compared to prepupal and adult stages for second instar grub, LT_{50} values against second instar grubs was 1.3 to 4.8 days.

Experiments were conducted to test the pathogenicity of the entomopathogenic fungus *B. bassiana* against all the developmental stages of Hadda beetle, *E. dodecastigma*. *B. bassiana* proved to be effective against all the stages except the eggs. In grubs and pupae 100 per cent mortality occurred after

120h and 168h after treatment with 10^8 spores/ ml. In adults, mortality was 100 per cent after 192h with the same dose (Gul and Hamid, 1999).

Higher doses of *B. bassiana* was found to be effective on later instars of *S. litura* (Dayakumar and Kanaujia, 2003). The pathogenicity of *B. bassiana* on eggs and pupae of lepidopteran pests *S. litura, S. obliqua* and *H. armigera* was investigated. Among these *S. litura* recorded the highest egg and pupal mortality (51.6 and 48.8%) (Pandey, 2003).

2.6.3.2. Metarhizium anisopliae Sorokin

In India, Sundarababu *et al.* (1983) studied the mass production and use of the fungus against coconut rhinoceros beetle, *Oryctes rhinoceros*. Aguda *et al.* (1987) reported the effectiveness of *M. anisopliae* against *Nilaparvatha lugens* on rice in Korean Republic. The treatment with $1.8x10^9$ conidia/ml of *M. anisopliae* caused 80 to 100 per cent mortality of all the five instars, prepupae and pupae of *H. armigera* within two to ten days (Gopalakrishnan and Narayanan, 1989). Sairabanu (2000) studied the usefulness of *M. anisopliae* in the control of diamond back moth on cauliflower. In pathogenicity studies 100, 90, 76.67 and 56.67 per cent mortality was recorded by spraying $1x10^8$ spores/ml of fungal suspension of *M. anisopliae* against first, second, third and fourth instar larvae of *H. armigera* respectively. LC_{50} value on second instar larvae of *H. armigera* was $1.18x10^5$ spores/ml (Wadayalkar *et. al*., 2003).

2.7. COMPATIBILITY OF ENTOMOPATHOGENS WITH CHEMICAL PESTICIDES AND BOTANICALS

2.7.1. Compatibility of *B. thuringiensis* with insecticides and botanicals

Most of the insecticides were compatible with *B. thuringiensis*, having little or no effect on spore germination or cell multiplication. Experiments conducted by Sutter *et al*. (1971) proved that low concentrations of carbamates (carbaryl and carbofuran) and organophosphates (diazinon, malathion and phorate) did not affect the bacterial growth, whereas others, especially the chlorinated hydrocarbons (DDT, aldrin and heptachlor) inhibited the growth.

Baskaran and Sekar (1976) reported that chemicals like DDT and fenthion significantly reduced bacterial population at all concentrations. Compatibility of *B. thuringiensis* with 27 chemicals revealed that carbamates were generally more compatible with *B. thuringiensis* (Morris, 1977).

According to Coventry *et al*. (1997) Neemazal- F and one per cent Neemazalpulvar solution (3000 ppm azadirachtin) inhibited the growth of *Bacillus cereus, Bacillus mycoides, B. thuringiensis* and *Bacillus subtilis*.

Monocrotophos and azadirachtin were reported compatible and safer for sporulation of *B. thuringiensis* (Pramanik *et al.,* 1997). They also reported that endosulfan significantly inhibited the growth of *B. thuringiensis* compared to other insecticides. Work done by Bhattacharya *et al.* (1998) showed that quinalphos 25 EC (0.05, 0.1 and 0.2%), endosulphan 35 EC (0.035, 0.07 and 0.14%), phosphamidon 85 SL (0.25 and 0.5%), ethion 50 EC (0.05 and 0.1%) and copper oxychloride 50 WP (0.2, 0.4 and 0.8%) inhibited the growth of the bacteria.

The chemical insecticides Cypermethrin (Patel and Vyas, 1999) and acephate (Sunildutt, 2000) did not affect the growth and sporulation of *B. thuringiensis*. Studies conducted by Maghodia and Vyas (2003) showed that among the insecticides tested, monocrotophos, methyl-o-demeton and azadirachtin were safe and compatible with *B. thuringiensis* while chlorpyriphos and quinalphos were compatible at half the field recommended doses only.

2.7.2. *B. bassiana*

The effect of carbaryl on the growth and sporulation of *B. bassiana* was studied by Aguda *et al*. (1988). They reported that all the five concentrations of the insecticide tested inhibited the germination of conidia. The insecticides *viz*., diazinon, primicarb, cypermethrin and oxamyl did not affect the growth of *B. bassiana* (Hopkanen, 1988).

Among the different botanicals tested neem oil and neem seed kernel extract were deleterious to germination of spore of *B. bassiana*. The insecticides *viz*.,

fenitrothion, primiphosmethyl, endosulfan and dicrotophos inhibited the germination and growth of *B. bassiana* (Malo, 1993).

Almeida *et al.* (1998) reported that the colony diameter of *B. bassiana* was significantly reduced with deltamethrin, methamidophos, cyhalothrin and endosulfan. The neonicotinoid insecticides, acetamiprid, imidacloprid and thiamethoxam were tested for their antifungal activity. The results showed that they had no negative effect on conidia germination, conidia production and vegetative growth of *B. bassiana* (Neves *et al.,* 2001). Experiments conducted by Sheila *et al*. (2003) evaluated the relative toxicity of the insecticides methylchlorpyriphos, disulfoton, ethion, methyl parathion and endosulfan to the fungus *B. bassiana.* Results indicated that endosulfan was moderately toxic, while ethion and disulfoton were selective to this fungus.

Essential oils of *Cymbopogon winterianus C. flexuous, Kaempferia galanga* and *C. martini* were tested for compatibility with fungi. All the fungi, *M. anisopliae, B. bassiana* and *Nomurea rileyi* exhibited complete inhibition of growth when exposed to essential oils. Among the plant extracts, *A. calanus* caused more than 50.0 per cent inhibition followed by *A. paniculata* (Levin, 2005).

2.7.3. ***M. anisopliae***

Carbaryl inhibited the conidial germination of *M. anisopliae* (Sundarababu *et al*., 1983 and Aguda *et al*., 1988). According to Aguda *et al*. (1984), the insecticides *viz*., monocrotophos, carbosulphan and azinphos ethyl inhibited spore germination of *M. anisopliae.* Neem oil at five per cent concentration inhibited germination and sporulation of *M. anisopliae* (Aguda and Rombach, 1986).

The insecticides *viz*., diazinon, primicarb, cypermethrin, oxamyl (Vanmien and Hokkanen, 1988), dichlorvos and hostathion (Moorehouse *et al.,* 1992) inhibited the growth of *M. anisopliae.* Nicotine sulphate and repellin inhibited the growth of *M. anisopliae*. But Neemark did not inhibit the growth. (Vyas *et al*., 1992). Morino and Alves (1998) reported that the chemicals chlorpyriphos and propetamphos reduced sporulation. The neonicotenoid insecticides acetamipride,

imidacloprid and thiomethoxam (Neves *et al*., 2001), monocrotophos, chlorpyriphos and azadirachtin (Gupta *et al*., 2002) were well tolerated by the fungus *M. anisopliae*.

2.8. INTERACTION OF MICROBIALS AND CHEMICALS

2.8.1. Interaction of *B. thuringiensis* and botanicals

Experiments conducted by Hellpap (1984) showed that a combination of neem seed kernel with *B. thuringiensis* increased the mortality of *S. frugiperda* larvae and considerably reduced the LT_{50} and LT_{100}. Justin *et al*. (1987) conducted experiments on third instar larvae of *P. xylostella* with *B. thuringiensis* alone and in combination with plant extracts and found that when *B. thuringiensis* was combined with neem seed kernel extract (5%), LC_{50} value was reduced by 1.9 times. Similarly the combination of *B.thuringiensis* with *Catharanthus roseus* (3%) resulted in a reduced LC_{50}. A marked increase was observed in the potency of *B. thuringiensis* endotoxin preparation against *S. littoralis*, when combined with plant products like orange and pomegranate peel (Salama and Sharaby 1988). The addition of potassium carbonate to dipel increased the larval mortality of *S. litura* (Salama *et al*., 1990).

According to Balasaraswathy (1990), leaf extracts of *Tagetus patula* and *Argemone mexicana* did not increase the potency of *B. thuringiensis* against *H. armigera* and *S. litura*. Spraying commercial formulation of *B. thuringiensis* and aqueous leaf extracts of three botanicals namely *A. indica, Ayapana triplinervis* and *L. camera* against *P. xylostella* nad *Crocidolomia binotalis* Zell in field cabbages. The botanicals had an enhancing influence on the *B. thuringiensis,* the combination treatment being more effective than the individual botanical or *B. thuringiensis* alone (Facknath, 1999).

According to Chatterjee and Senapati (2000) joint spraying of *B. thuringiensis* (0.05%) + azadirachtin 1500 ppm (0.15%) recorded the best result of 87.43 and 84.85 per cent reduction in larval population of *H. armigera* after three and fourteen days of sprayings respectively. Sailaza and Krishnayya (2003) reported that the treatments *B. thuringiensis kurstaki* (*B.t.k*) 0.2%+neem oil 0.1 %,

B.t.k 0.2 %+ neem oil 0.1 % were more effective against *S. litura, C. binotalis* and *P. xylostella* resulting in highest per cent reduction of larval population (63 to 71, 57 to 69,and 49 to 57, respectively). The effects of bacterial toxins (*B. thuringiensis*) and botanical insecticides (*A. indica* and *V. negundo*) on Lactate dehydrogenase (LDH) activity in *C. medinalis* were evaluated. Both the combination treatments (*A. indica* + *B. thuringiensis* and *V. negundo* + *B. thuringiensis*) at sublethal levels resulted in higher mortality by severely inhibiting LDH activity (Nathan *et al*., 2006). Application of *B.t.k* 0.2 per cent +neem oil 5 per cent, *B.t.*k 0.2 per cent + citronella oil 5 per cent, *B.t.k* 0.2 per cent + karanj oil 5 per cent, *B.t.k* 0.2 per cent + cotton seed oil 5 per cent and *B.t.k* 0.2 per cent + sesamum oil 5 per cent resulted in significantly higher larval mortality (80 to 55%), feeding inhibition (80 to 32.4%), mean larval weight reduction(30 to 22.2%), pupal weight reduction (31 to 15.1%), prolonged larval and pupal periods (11 to 9.3 days, 12 to 10.3 days respectively) over untreated check (Babu *et al*., 2007). Combinations of sublethal concentrations of Bt (*B. thuringiensis*) spray formulation with azadirachtin at EC_{50} or EC_{95} levels not only enhanced the toxicity but also reduced the duration of mortality when used in combination. Bt – azadirachtin combinations of LC_{50} + EC_{20} and LC_{50} + EC_{50} result in 100 per cent mortality (Singh *et al*., 2007).

2.8.2. Interaction of *Bacillus thuringiensis* and chemicals

Dabi *et al.* (1988) evaluated the efficacy of Dipel alone and in combination with sublethal doses of carbaryl (0.05%), monocrotophos (0.02%) and phenthoate (0.02%) against third instar larvae of *H. armigera*. The combinations had a synergestic action, the most effective were Dipel + endosulfan and Dipel + monocrotophos.

Among the bacterial formulations and plant extract combination, Delfin + *V. negundo* recorded the highest mortality against *H. armigera* (Levin, 2005).

2.8.3. Interaction of nuclear polyhedrosis virus and botanicals

There was an additive effect when NPV was combined with nicotine sulphate and Pyrethrum against *S. litura* larvae (Choudhari and Ramakrishnan, 1983) and with methanol extract of *O. sanctum* and *A. calamus* agaist *H. armigera* and *S. litura* (Devaprasad, 1989). Work done by Rabindra *et al*. (1991) showed that a combination of NPV + *V. negundo* was significantly more effective than virus alone in reducing the attack of *H. armigera*. Aqueous leaf extract (10%) of *V. negundo* when applied with Ha NPV at 1.5 * 10^{12} POB / ha gave better control of *H. armigera* and increased the grain yield (Rabindra and Jayaraj, 1992). Extracts of *O. santum*, *A. calamus* and *Allium sativum* (garlic) in methanol, petroleum ether and acetone either alone or in combination with NPV were bioassayed against third instar larvae of *S. litura*. Combinations with garlic resulted in highest larval mortality (Prasad *et al*., 1993). The potential enhancement of azadirachtin and its impact on *H. armigera* was evaluated in the laboratory. Combination of NPV with azadirachtin significantly reduced the consumption index and decreased the efficiency of conversion of ingested food (ECI) to 5.07 per cent and efficiency of conversion of digested food (ECD) to 9.8 per cent (Kumar and Murugan, 1998 and 1999).

A synergestic effect of azadirachtin and Sl NPV (Spodavax) at sub lethal levels was reported by Nathan *et al*. (2004). The combination NPV+*V. negundo* recorded the highest mortality percentage in NPV + plant extract combinations against *H. armigera*. Among the NPV+essential oil combinations, NPV+*K. galanga* recorded the highest mortality percentage followed by NPV + *C. martini*, *NPV +C. flexuosus* and NPV + *C. winterianus.* The combination involving essential oil also enhance the effectiveness in bringing high mortality and reduce the LT_{50} as much as 6.5 to 4.2 days (Levin, 2005). Efficacy of combination of nucleopolyhedrosis virus and azadirachtin on *S. litura* was studied by Nathan and Kalaivani (2006). They reported that when consumed together (AZA + NPV) larvae died significantly faster compared with larvae that consumed NPV or AZA alone.

2.8.4. Interaction of entomopathogenic fungi and botanicals

Among the different combinations of botanicals with *N. rileyi* Samson ($2*10^{11}$ conidia / ha) + NSKE (5%), *N. riley* ($2*10^{11}$ conidia / ha) + *V. negundo* (5%) proved to be as effective as recommended insecticides in reducing larval incidence of *S. litura* (Patil *et al*., 2003).

The entomopathogenic fungi *Paecilomyces fumosoroseus* in combination with azadirachtin resulted in highest nymphal mortality (90%) when tested against white fly *Bemisia argentifolii* (James, 2003).

In the laboratory bioassay of entomopathogenic fungi and plant extract combinations, *N. rileyi + V. negundo, M. anisopliae + V. negundo* and *B. bassiana +V. negundo* recorded the maximum mortality percentage when tested against *H. armigera*. (Levin, 2005). An experiment was done by Mohan *et al*., 2009 to test the influence of neem formulations on growth of *B. bassiana* and to evaluate their compatibility and synergism. About 30 isolates of *B. bassiana* were screened for their compatibility and in that 23 isolates were found compatible with neem. The combined treatment of *B. bassiana* with neem was found to have a synergestic effect on *S. litura* mortality when *B. bassiana* isolate compatible to neem was used and the effect was antogonostic with an isolate sensitive to neem.

Materials and Methods

3. MATERIALS AND METHODS

The investigation on "Biopotency of Indian Privet, *Vitex negundo* Linn. (Verbenaceae) against *Spodoptera litura* F. (Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* F. (Coleoptera: Coccinellidae)" was conducted in the Department of Entomology at College of Horticulture, Vellanikkara during March 2005 to February 2008. The materials used and the methods followed are described here.

3.1. REARING OF TEST INSECTS

3.1.1. *Spodoptera litura*

The culture of *S. litura* was maintained in the laboratory both on natural food (castor and banana) and on the semi-synthetic diet as standardised by Ballal, (2004).

Larvae of *S. litura* were initially collected from banana fields of College of Horticulture, Vellanikkara and Banana Research Station, Kannara and were further mass reared in the laboratory.

3.1.1.1. Rearing of S. litura on natural hosts – castor and banana

Larvae of *S. litura* were reared separately on castor and banana leaves kept inside separate plastic jars (1.5 litre capacity) closed with muslin cloth. Fresh leaves were given till the pupation of the larvae. Pupae were collected and sorted out for their sexes. The male and female pupae were differentiated based on the distance between the genital and anal pores. This distance was more than double in female than that in male. Another distinguishing character of the female pupa, was that on either side of the genital pore, a "V" shaped depression or fold extending upto the tenth segment was visible. Male and female pupae were kept in folds of tissue paper in a Petri dish placed inside a plastic bucket of two litre capacity @ eight pairs per bucket. Mouth of the bucket was then covered with a muslin cloth. Adult moths emerged within a period of 9-10 days. Cotton pieces soaked in honey solution fortified with few drops of vitamin E were placed

on the sides of the bucket for the emerging adults. Folded paper sheets were kept inside the bucket as substrate for egg laying. Egg masses laid on the folded papers were collected and surface sterilized with 0.05 per cent Sodium hypochlorite (Na O Cl) solution for five minutes, thoroughly washed with distilled water to remove traces of chemical and then dried. The egg masses were then kept inside plastic jars containing fresh castor or banana leaves and the rearing was continued for getting a steady supply of larvae for different experiments (Plates 2b & 2a).

3.1.1.2. Rearing of S. litura on semi-synthetic diet

Larvae were also reared on semi-synthetic diet as per the method of Ballal, (2004).

3.1.1.2(a). Composition of semi-synthetic diet for rearing S. litura

Sl.No.	Name of the ingredients		Quantity
1	Chick pea flour	A	105 g
2	Methyl para hydroxy benzoate		2 g
3	Sorbic acid		1 g
4	Yeast tablets		10 g
5	Agar – agar	**B**	12.75 g
6	Ascorbic acid	C	3.25 g
7	Multi vitaplex		2 caps
8	Vitamin E		2 caps
9	Streptomycin sulphate		0.25 g
10	Formalin (10 %)	**D**	5 ml
11	Water		780 ml

3.1.1.2(b). Method of preparation of semi-synthetic diet

Part A of the diet was mixed with 390 ml water and ground well in a blender for two minutes. Agar was separately boiled in 390 ml of water and added

to Part A in the blender and mixed thoroughly. Part C and Part D were added separately and the blender was operated for two minutes each. The hot diet @ 20 ml was poured into sterilized beakers of 50 ml capacity and allowed to solidify. After solidification, the beakers were covered with muslin cloth and kept in a refrigerator and used as when required.

3.1.1.2(c). Method of rearing S. litura on semi-syntheticdiet

Second instar larvae collected from natural food were released @ two larvae per beaker containing semi-synthetic diet and closed with muslin cloth (Plate 2c). The diet was changed at an interval of two days upto third instar stage and daily for later instars. The larvae were allowed to pupate and the pupae were collected from the diet, cleaned well and continued rearing as mentioned in 3.1.1.1.

3.1.2. Rearing and maintenance of *Henosepilachna vigintioctopunctata*

The culture of the second test insect, *H. vigintioctopunctata* was maintained on brinjal leaves in the laboratory. Grubs collected from the field were used to establish the nucleus culture of *H. vigintioctopunctata*. They were reared on fresh brinjal leaves kept in Petri dishes of 9 cm diameter. Moistened filter paper discs were kept on the bottom of the Petri dishes to keep the leaf turgid. Fresh leaves were introduced daily to the Petri dish after removing leaf debris and excreta. Immediately after the adult emergence, they were sexed based on the genital pore (for females, genital pore will be more clear and visible than that in males). The paired adults were then confined in separate Petri dishes @ two pairs per Petri dish for breeding and oviposition. Cotton swabs soaked with 15 per cent honey solution fortified with vitamin E were kept inside the Petri dishes for the adults to feed on. Egg masses were collected daily and kept in separate Petri dishes for hatching. When the grubs reached second instar stage they were transferred to tender brinjal leaves. Required number of grubs and adults were drawn from the culture whenever needed for experiments (Plates3a,3b,3c and 3d).

1a. Leaves

1b. Shoots

1c. Flowers

Plate1. *V. negundo* parts tested for biological efficiency

2a. Banana

2b. Castor

2c. Semi-synthetic diet

Plate 2. Rearing of *Spodoptera litura* on different hosts

3.2. PREPARATION OF EXTRACTS FROM LEAVES, FLOWERS AND SHOOTS OF *VITEX NEGUNDO* WITH DIFFERENT SOLVENTS

Fresh leaves, shoots and flowers of *V. negundo* (Plates 1a, 1b & 1c) were collected from medicinal plant garden, College of Horticulture, Vellanikkara. Acetone, hexane, methanol and water extracts of the three parts were obtained.

3.2.1. Processing of plant materials and preparation of different concentrations of extracts

Only the middle leaves and shoots were used for extraction. The plant materials were washed and chopped into small pieces before maceration. Fifty gram each of the plant material was macerated separately with 100 ml each of acetone, hexane, methanol and water separately in an electric blender. The macerated slurry was first strained through a muslin cloth, then filtered through a Whatman No.1 filter paper in a funnel and collected in a volumetric flask. The volume was made upto 100 ml with respective solvents to form the primary stock solution of the extract.

3.3. BIOEFFICACY OF DIFFERENT EXTRACTS OF *V.NEGUNDO*

The bioefficacy of four different solvent extracts (acetone, hexane, methanol and water) from three different plant parts (leaves, shoots and flowers) of *V. negundo* was tested against two insect pests *viz., S. litura and H. vigintioctopunctata*. The part of *V. negundo* that showed highest bioactivity was identified and further experiments were carried out with that.

3.3.1. Biological efficacy of *V. negundo* against *S. litura*

The experiment was conducted in a factorial CRD consisting of three factors viz., three plant parts (leaves, shoot and flowers), four solvent extracts (acetone, hexane, water and methanol) and four concentrations (1, 2, 4 and 6%). Alltogether, there were 48 treatments with three replications for each. Third instar larvae (5 days old) of *S. litura* were used for the study. Fresh castor leaves were collected from the field washed and dried. Circular leaf discs of 5 cm diameter were cut from freshly collected castor leaves and dipped for one minute in

3a. Egg mass

3b. Grub

3d. Adult

3c. Pupae

Plate 3. Rearing of *Henosepilachna vigintioctopunctata* on brinjal

different concentrations of 1, 2, 4 and 6 % of *V. negundo*. The leaf discs were air dried and placed over moist tissue paper kept in Petri dishes of 9 cm diameter. Ten third instar larvae of *S. litura* were released over the treated leaf discs of each treatment and allowed to feed for 24 hours. The larvae were then transferred and allowed to feed on fresh untreated castor leaves for a period of seven days. Observations on mortality, morphogenic malformations of the larvae were recorded at an interval of 24 hours. Malformed and moribund larvae were also considered as dead ones.

3.3.2. Biological efficacy of *V. negundo* against *H. vigintioctopunctata*

Leaves of brinjal were used for preparing leaf discs for the different treatments**.** Third instar grubs of *H. vigintioctopunctata* were used for the study and the experiment was conducted following the same method described in 3.3.1.

3.4. MODE OF ACTION OF DIFFERENT SOLVENT EXTRACTS OF *V. NEGUNDO* AGAINST *S. LITURA* AND *H. VIGINTIOCTOPUNCTATA*

The extracts of the plant part that showed highest bioefficacy in 3.3 was selected for studying the different mode of actions of *V. negundo* on *S. litura* and *H. vigintioctopunctata.*

3.4.1. Ovipositional deterrency

Experiments were conducted to determine the ovipositional deterrent action of different solvent (acetone, hexane, methanol and water) extracts prepared from the most bioeffective part of *V. negundo* against *S. litura* and *H. vigintioctopunctata.*

3.4.1(a). Ovipositional deterrent action of different solvent extracts of *V. negundo* against *S. litura*

Fresh castor leaves with shoots collected from the fields were taken to the laboratory and kept in conical flasks containing water. Mouth of the conical flask was then plugged with cotton and the conical flask was covered with a bell jar

which acted as an oviposition chamber for the adult moths of *S. litura* (Plate 4a). Cotton pieces soaked in 15 per cent honey solution fortified with vitamin E was kept in the oviposition chamber to serve as food for the adult moths of *S. litura*. Four concentrations viz., 1, 2, 4 and 6% of each of the solvents – acetone, hexane, methanol and water extracts prepared from the leaves of *V. negundo* were sprayed to run off stage on castor leaves using a hand atomizer and allowed to dry. One pair of freshly emerged adult moths of *S. litura* were released inside each jar(Plate 4b). For each treatment, ten replications were maintained and untreated controls were also maintained. Observations on egg laying were recorded for each treatment after a period of 72 hours of treatment.

3.4.1(b). Ovipositional deterrent action of different solvent extracts of *V. negundo* against *H. vigintioctopunctata*

Fresh and clean brinjal leaves of uniform size and age, collected from the field were sprayed with different solvent extracts prepared from the leaves of *V. negundo* at various concentrations (1, 2, 4 and 6 %). After drying the treated leaf was placed inside a Petri dish of 90 cm diameter @ one leaf per Petri dish. Cotton swabs soaked in 15 per cent honey solution fortified with vitamin E placed at the two ends of the leaf served as food for the adult beetles. A pair of adult *H. vigintioctopunctata* beetle was released on the treated leaves @ one pair per Petri dish. Ten replications were maintained for each treatment. Leaves sprayed with water, acetone, hexane and methanol served as untreated control. Number of eggs laid in each treatment was recorded after a period of 72 hours.

3.4.2. Ovicidal action of leaf extracts of *V. negundo*

3.4.2(a). Ovicidal action of different solvent extracts of *V. negundo* against *S. litura*

One day old egg masses of *S. litura* collected from the stock culture were used for the determination of ovicidal action of acetone, hexane, methanol and water extracts of *V. negundo*. Scales on the egg masses of *S. litura* were removed by a camel hair brush to make it easier for counting and exposing them for

4a. Ovipositional chambers

4b. Adult moths inside the ovipositional chambers

Plate 4. Ovipositional deterrency of *V. negundo* against *S. litura*

treatment. Precounted eggs in an egg mass along with the leaf substrate were dipped in different solvent extracts at four concentrations (1, 2, 4 and 6%) for one minute. The egg masses were air dried and kept for hatching for two days on moist blotting paper in a Petri dish. One egg mass was kept per treatment and each treatment was replicated thrice. Eggs dipped only in water, acetone, methanol and hexane served as control for the different solvents. After two days of the treatment, unhatched eggs were counted and the hatching percentage was worked out as follows.

$$\text{Hatching percentage} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs treated}} \times 100$$

3.4.2(b). Ovicidal action of different solvent extracts of *V. negundo* against *H. vigintioctopunctata*

The ovicidal action of *V. negundo* against *H. vigintioctopunctata* was also studied using one day old egg masses of *H. vigintioctopunctata* collected from the stock culture maintained in the laboratory. Number of eggs in each egg mass was counted prior to the application of the treatment and the egg mass along with the leaf substrate was dipped in leaf extracts prepared using acetone, hexane, water and methanol at various concentrations (1, 2, 4 and 6%) for one minute. After air drying, the egg mass was kept for hatching in a Petri dish containing moist tissue paper. Number of egg masses used for treatment, period kept for hatching, number of replications, control treatments and observations were same as described in 3.4.2. (a).

3.4.3. Effect of *V. negundo* on growth and development of *S. litura*

3.4.3.1. Effect of different solvent extracts of V. negundo on growth and development of S. litura

The effect of *V. negundo* leaf extracts prepared with acetone, methanol, hexane and water on the growth and development of *S. litura* larvae (third instar) was studied by feeding the larvae on treated castor, banana and artificial diet. Four

concentrations, *viz*., 1, 2, 4 and 6 per cent for each solvent extracts were prepared and applied on fresh leaves of castor and banana and also on artificial diet. Ten larvae (third instar) were released in each treatment and allowed to feed on the treated food (castor, banana and artificial diet) for a period of 24 hours. The larvae were then transferred to respective untreated food and maintained till their pupation. There were three replications for each treatment, and an untreated control was also maintained.

Observations on percentage pupation, weight of pupae and duration of third instar larvae in different treatments were recorded.

3.4.4. Antifeedant action

3.4.4.1. Antifeedant action of different solvent extracts of V. negundo against S. litura

Solvent extracts of *V. negundo* (acetone, methanol, hexane and water) at different concentrations (1, 2, 4 and 6%) were prepared. Circular pieces of 5cm diameter were cut from banana and castor leaves of uniform age and weighed and then dipped in the different concentrations of extracts for 1 minute. After air drying, each leaf disc was placed on a filter paper kept over a wet padding of tissue paper in a Petri dish. Fourth instar *S. litura* larvae @ one larva per treatment prestarved without food for four hours, were weighed and released on the leaf discs in different treatments. In case of artificial diet, circular pieces of diet were taken with the help of a cork borer and weighed before the treatment and treated diet was fed to *S. litura*. Ten replications were maintained for each treatment. Leaf discs treated with solvents alone served as control. The larvae were allowed to feed on treated food for 24 hours and their weights were taken after 24 hours. Weight of unfed leaves and diet and excreta of larvae were also taken to calculate different nutritional indices.

Percentage of leaf protection by the botanical preparations = $\frac{A - B}{A} \times 100$

A = Weight of the leaf consumed in the control

B = Weight of the leaf consumed in the treatment

3.4.4.2. Antifeedant action of V. negundo extracts on H. vigintioctopunctata

The antifeedant action of *V. negundo* on *H. vigintioctopunctata* was also studied. Fourth instar grubs of *H. vigintioctopunctata* were used for the experiment. Brinjal leaves of uniform age and size were weighed and dipped in different concentrations of extracts and dried. Fourth instar grubs pre-conditioned without food for four hours were weighed and released for feeding in each treatment. The uneaten portions of leaves after 24 hours of exposure and the grubs were weighed. Untreated control was also maintained and ten replications were there for each treatment. The difference between the pre-treatment and post feeding weights gave the weight of the leaf consumed. Weight of leaves and faecal matter were also recorded and different nutritional indices were calculated.

3.4.5. Larval starvation

The larval starvation as influenced by different solvent extracts of *V. negundo* was calculated for both *S. litura* and *H. vigintioctopunctata* based on the experiment conducted in 3.4.3.1. as per Saradamma (1989)

$$\text{Percentage of larval starvation} = \frac{(C-E)}{(C-S)} \times 100$$

C = Mean weight gain of larvae in control after 24 hours

E = Mean weight gain of larvae in the treatment after 24 hours

S = Mean weight loss of starved larvae in 24 hours (the figure is negative)

3.4.6. Food consumption and utilization indices

The effect of different solvent extracts of *V. negundo* on the nutritional indices of *S. litura* and *H. vigintioctopunctata* was studied by calculating the following indices (Wald-bauer, 1968).

(a) Approximate Digestibility (AD) = $\frac{\text{Weight of food ingested – weight of faeces}}{\text{Weight of food ingested}}$

(b) Efficiency of conversion of ingested food to body substance (ECI)

$$ECI = \frac{\text{Weight gained}}{\text{Weight of food ingested}} \times 100$$

(c) Efficiency of conversion of digested food to body tissue (ECD)

$$ECD = \frac{\text{Weight gained}}{\text{(Weight of food ingested- Weight of faeces)}} \times 100$$

All the above indices were calculated on fresh weight basis for both *S. litura* and *H. vigintioctopunctata*.

3.4.7. Juvenomimetic activity

3.4.7(a). Juvenomimetic action of different solvent extracts of *V. negundo* against *S. litura*

Teepol 0.1 per cent was added to the extracts as a wetting agent. The solvent extracts were applied topically on the abdominal tergites of the newly moulted last instar larvae *S.litura* using a Hamilton micro applicator. Ten larvae were used for each treatment. Extracts were applied @ 2 µl per larvae and were confined in Petri dishshes (9 cm). Two µl each of the solvent alone applied on ten larvae served as control and all the treatments were replicated three times. After one hour, the treated larvae were transferred to Petri dishes containing fresh castor, banana leaves and synthetic diet and observed till adult emergence. Observations on total pupation, malformed pupae, total adult emergence and malformed adults were taken at daily intervals.

3.4.7(b). Juvenomimetic activity as influenced by different solvent extracts of *V. negundo* against *H. vigintioctopunctata*

The experiment method as described in 3.4.7.(a) was conducted for *H. vigintioctopunctata* also. Last instar grubs (fifth instar) were used for the study and the treated grubs were transferred to fresh brinjal leaves kept in petridishes of 9 cm. Observations on total pupation, malformed pupae, total adult emergence and malformed adults were taken at daily intervals.

3.4.8. Effect of *V. negundo* extracts on longevity

3.4.8(a). Influence of different solvent extracts of *V. negundo* on *S. litura*

Acetone, hexane, water and methanol extracts of *V. negundo* were applied topically at four concentrations (1, 2, 4 and 6%) on the last instar larvae of *S. litura* as described in 3.4.7.a. observations on the date of adult emergence and death were recorded and the longevity of *S. litura* was calculated.

3.4.8(b). Longevity of *H. vigintioctopunctata* adults as influenced by different solvent extracts of *V. negundo*

The experiment detailed in 3.4.8 (a) was repeated with last instar grubs of *H. vigintioctopunctata* also. Observations on the date of emergence and death of adult beetles were recorded to calculate longevity.

3.4.9. Influence on fecundity

Fecundity denotes the capacity of an adult female to lay eggs. An experiment was conducted to understand effect of different solvent extracts of *V. negundo* on fecundity of the two insects.

3.4.9(a). Influence of different solvent extracts of *V. negundo* on fecundity of *S. litura*

The effect of 1, 2, 4 and 6% concentration each of acetone, hexane, water and methanol extracts of *V. negundo* was studied after their topical application (see 3.4.7.a) on last instar of *S. litura*. Ten larvae were maintained in each

treatment and three replications were maintained along with untreated control. Pupae were sexed as per section 3.1.1.1. The adults after emergence were transferred to plastic buckets of two litre capacity @ 1 pair per bucket and the open end was covered with a muslin cloth. Diluted honey (15 %) fortified with vitamin E served as food for the adult moths. Folded papers were kept inside the bucket for egg laying. The number of eggs laid by the adult females were counted daily to determine the fecundity in different treatments.

3.4.9(b). Influence of different solvent extracts of *V. negundo* on fecundity of *H. vigintioctopunctata*

Last instar grubs of *H. vigintioctopunctata* were used for the study. The experiment detailed in 3.4.8 (a) was repeated for Epilachna beetles also with ten grubs and three replications per treatment. The emerged adults in different treatments were sexed and confined in petridishes of 9cm diameter containing fresh brinjal leaves @ one pair per petridish. Honey solution (15 %) fortified with vitamin E served as food for the adult beetles for egg laying. Fecundity in different treatments were recorded by counting the number of eggs laid by the adult female beetle in each treatment.

3.5. BIOASSAY TO ESTIMATE MEDIAN LETHAL DOSE (LD_{50}) OF DIFFERENT SOLVENT EXTRACTS OF *V. NEGUNDO*

3.5.1. Bioassay of solvent extracts of *V. negundo* against *S. litura*

Solvent extracts of *V. negundo* (acetone, hexane, water and methanol) were prepared at one to ten per cent concentrations. Teepol 0.1 per cent was added to the extracts as a wetting agent. Two µl of the treatments were topically applied on the thoracic tergal plate of the freshly moulted third instar larvae of *S. litura* using Hamilton micro applicator. Ten, third instar larvae were maintained per treatment and each treatment was replicated three times. The treated larvae were then reared on semi-synthetic diet and larval mortality was recorded after 24 hours and the observations continued upto a period of 120 hours. LD_{50} was calculated by Probit analysis (Finney, 1971) using SPSS programme.

3.5.2. Bioassay of solvent extracts of *V. negundo* against *H. vigintioctopunctata*

Freshly emerged adult beetles collected from the stock culture of *H. vigintioctopunctata* were used for the study. After the adults were treated with different concentrations as described in 3.5.1. they were transferred to fresh brinjal leaves kept in Petri dishes of 9 cm diameter. Adult mortality in different treatments were recorded at 24 hours interval upto a period of 120 hours, and LD_{50} was calculated.

3.6. INSECTICIDAL ACTION OF DIFFERENT SOLVENT EXTRACTS OF *V. NEGUNDO*

3.6.1. Insecticidal action of different solvent extracts of *V. negundo* against *S. litura*

Freshly emerged third instar larvae of uniform size and age were collected from the mass culture maintained in the laboratory. Solvent extracts of *V. negundo* (acetone, hexane, water and methanol) were prepared at different concentrations (1, 2, 4 and 6%). Teepol 0.1 per cent was added to the extracts as a wetting agent. Ten insects were taken for each treatment and three replications were maintained for each treatment. The different concentrations of the solvent extracts were directly sprayed using an atomizer on the test insects released in clean Petri dishes. Larvae sprayed with distilled water containing teepol 0.1 per cent served as control. After air drying, the larvae were transferred to beakers (50 ml capacity) containing semi synthetic diet. Mortality counts were taken at daily intervals and the observations continued upto a period of 120 hours.

3.6.2. Insecticidal action of different solvent extracts of *V. negundo* against *H. vigintioctopunctata*

The experiment was conducted as detailed in section 3.6. (a), for Epilachna beetles also. Freshly emerged adult beetles were used in treatments and the treated adults were transferred to fresh brinjal leaves kept in Petri dishes (9 cm).

Observations on adult beetle mortality were recorded at daily intervals for a period of 120 hours.

3.7. COMPATIBILITY OF *V. NEGUNDO* EXTRACTS AND ENTOMOPATHOGENS

Compatibility studies were conducted to find out whether *V. negundo* extracts have any inhibitory effect on the entomopathogens viz., *Metarhizium anisopliae* var. *anisopliae*, *Beauveria bassiana* Balsamo and *Bacillus thuringiensis* var. *kurstaki*.

Pure cultures of *M. anisopliae* and *B. bassiana* were obtained from Project Directorate of Biological Control (PDBC), Bangalore and were maintained on PDA medium in the laboratory. *B. thuringiensis* was used in the form of commercial formulation Delfin. *V. negundo* leaf extracts in acetone, hexane, methanol and water solvents prepared at six per cent concentration were used for the study. Two chemical insecticides (quinalphos and cypermethrin) and a botanical insecticide (1% Azadirachtin) were also included for comparison. All together there were eight treatments including control with three replications and the details are given below.

Treatments	Dose
T1 – quinalphos (Ekalux 25 EC)	0.05%
T2 - cypermethrin (Ralothrin 10EC)	0.002%
T3 - 1% Azadirachtin (Econeem plus)	0.03%
T4 – Methanol extract	6%
T5 – Acetone extract	6%
T6 – Hexane extract	6%
T7 - Water extract	6%
T8 - Control	

The experiment was conducted by poison food technique proposed by Vincent (1927). Actively growing twelve days old cultures of *M. anisopliae* and

B. bassiana were used for the study. Hundred ml conical flasks having 100 ml Potato Dextrose Agar (PDA) media (sterilized) were taken, the different extracts were mixed with the media aseptically, the flasks were shaken well and the media was poured into six sterilized petriplates each. Four mm disc of fungal mycelia taken from 12 days old culture, using sterilized cork borer was kept at the centre of the media in Petri dishes using a sterilized needle. The dishes were incubated at room temperature and observed daily for fungal growth. The diameter of the colony was recorded daily. Control was also maintained without adding the extracts.

3.7.1. Preparation of leaf extracts

Fresh leaf samples were collected and weighed. A known quantity of the plant materials (0.6 g) were surface sterilized with 70 per cent ethanol. They were washed and kept for air drying. Then the leaves were chopped into small pieces and macerated in a sterlilised mortar and pestle with 10 ml of respective solvents/ sterile water. The extract was then filtered through a muslin cloth and added to the conical flask containing the sterilized medium. It was mixed well and transferred to the petriplates aseptically. Four mm disc of fungal mycelia taken from 12 days old culture, using sterilized cork borer was kept at the centre of the media in Petri dishes using a sterilized needle. The dishes were incubated at room temperature and observed daily for growth. The diameter of the fungal growth was recorded daily.

The per cent inhibition of fungal growth was calculated by using the formula suggested by Vincent (1927).

$$I = (C-T/C) \times 100$$

I = Per cent inhibition of growth

C = Diameter of fungal growth in control

T = Diameter of fungal growth in treatment

3.7.2. Inhibitory effect of *V. negundo* solvent extracts on the growth of *B. thuringiensis*

The inhibitory effect of different solvent extracts of *V. negundo* on *B. thuringiensis* was studied by filter disc bio assay.

3.7.2.1. Filter disc bioassay

One hundred ml of beef extract agar (T3) medium (peptone 10 g, beef extract 5g, agar 20g and distilled water 1 litre) was prepared in 250 ml conical flask and sterilized. The different treatments as cited in the section 3.7.1. Delfin was added to the media @ 0.2 g per 100 ml of the media. The required quantity of chemicals or *V. negundo* extracts were added aseptically to 100 ml medium to get the required concentration. The media and *B. thuringiensis* formulation were mixed well and transferred into sterile Petri dishes. The dishes were then incubated for about 24 – 48 h. For each concentration, three replications were maintained. Treatments were same as explained above in section 3.7.1. Sterile filter paper of 4 mm diameter was dipped in respective insecticides / botanicals and dried over a sterile wire net. The dried filter paper disc was placed on the T3 medium inoculated with pure culture of *B. thuringiensis* using sterile forceps. The Petri dishes were incubated at room temperature. Another filter paper disc dipped in sterile water served as control. The diameter of the inhibition zone developed around the filter paper disc dipped in different treatments was recorded for one week.

3.8. JOINT ACTION OF SOLVENT EXTRACTS OF *V. NEGUNDO* AND ENTOMOPATHOGENS

Bioefficacy of different solvent extracts of *V. negundo* individually and in combination with the entomopathogens viz., *M. anisopliae, B.bassiana* and *B. thuringiensis* were evaluated under laboratory condition. This study was conducted to assess the synergestic or antagonistic response in the combined application of solvent extracts of *V. negundo* and entomopathogens in the management of *S. litura* and *H. vigintioctopunctata*.

3.8.1. Pathogenicity of *M. anisopliae* and *B. bassiana* on *S. litura* and *H. vigintioctopunctata*

Two entomopathogenic fungi, namely *M. anisopliae* and *B. bassiana*, @ $1x10^8$ spores/ ml were initially assayed for their pathogenicity against third instar larvae of *S. litura* and *H. vigintioctopunctata*. This was done to ascertain the virulence of the fungi on the test insects. Ten third instar larvae of *S. litura* and *H. vigintioctopunctata* were first inoculated with *M. anisopliae* and *B. bassiana* and after a few days the fungi were reisolated from the diseased cadavers showing typical mycosis. After reisolation from the cadavers, the isolates were purified by subculturing on PDA from where the conidia for the bioassay were harvested from the twelve days old cultures just before use by washing from the surface of the plates with 100 ml of sterile distilled water containing 0.1 % teepol. Conidial suspensions were standardized for each fungal isolate with haemocytometer using the following formula (Mark and Douglas, 1997).

Number of spores / ml = X x 400 x 10 x 1000x D/Y

Where X = Number of spores counted totally

Y = Number of smaller squares counted

10 = Depth factor

1000 = Conversion factor for mm^3 to cm^3

D = Dilution factor

Newly moulted third instar larvae/ grubs of *S. litura* and *H. vigintioctopunctata* were bioassayed for their susceptibility to the different fungal isolates. Concentrations used were 10^6, 10^7, 10^8 and 10^9 spores per ml. Ten larvae taken in a Petri dish lined by a filter paper were directly sprayed with conidial suspension using a hand atomizer. Three such replications were maintained for each concentration. Control insects received a spray of only teepol in sterile distilled water. After air drying, the treated larvae were carefully transferred into individual beakers (50 ml capacity) containing freshly prepared semi synthetic diet (without formalin). Formalin being a sterilizing agent having antifungal effect, its use was avoided while preparing the diet. The larvae were reared in the diet till pupation and per cent mortality was recorded at 24 h interval.

3.8.1.1. Joint action of M. anisopliae and V. negundo extracts

The synergistic / antagonistic interaction of *V. negundo* extracts with *M. anisopliae* against *S. litura* was studied. The individual effect of acetone, hexane, methanol and water extracts and *M. anisopliae* and their joint effect on *S. litura* were bioasayed. Ten third instar larvae of *S. litura* were used in each treatment and was replicated thrice. For joint action the larvae were first treated with a lower concentration (3%) of different solvent extracts followed by direct spraying with *M. anisopliae* (0.5×10^8 spores / ml). The treated larvae were reared on synthetic diet till pupation. Mortality percentage was taken at 24 hours interval. Larval mortality due to *M. anisopliae* was ascertained by microscopic examination of the dead larvae. LT_{50} values were also calculated.

The same experiment was conducted against *H. vigintioctopunctata* grubs also. Third instar grubs were used for the study. Treated grubs were transferred to fresh brinjal leaves. Observations on mortality were recorded at 24 hours interval and continued upto 10 days.

3.8.1.2. Joint action of B. bassiana and V. negundo extracts

The experiment was conducted against two test insects viz., *S. litura* and *H. vigintioctopunctata***.** The same procedure as detailed in section 3.8.1.1 was followed here also. The sub lethal concentration of the fungal suspension @ 0.5×10^8 spores /ml and *V. negundo* extracts @ three per cent were used in this study. The per cent mortalities in different treatments were calculated. Observations were continued upto ten days.

3.8.2. Joint action of *V. negundo* extracts with *B. thuringiensis*

The individual effect of different solvent extracts, *B. thuringiensis* and the joint action of leaf extracts with *B. thuringiensis* were tested against third instar larvae of *S. litura*. Solvent extracts were directly sprayed on the larvae kept in Petri dishes (9 cm) and *B. thuringiensis* was added and fed to the larvae. For each treatment, 10 larvae were used and the treatments were replicated thrice. Observations on mortality were recorded at 24 hours interval.

3.8.3. Joint action of NPV and extracts of *V. negundo* against *S. litura*

Culture of SL NPV, was obtained from PDBC, Bangalore. Initially NPV was applied alone at the recommended dose ($1.5x10^9$ POB/ml) against third instar larvae of *S. litura.* The NPV isolate at 1.5×10^9 was prepared after enumeration with haemocytometer. Ten microlitre of the viral suspension was taken and uniformly smeared to the diet with a blunt end of polished glass rod and ten third instar larvae of uniform size were released on the diet 15 minutes after surface treatment.The treatments were replicated thrice. The larval mortalities were recorded at 24 hours interval.

The individual action of solvent (acetone, hexane, water and methanol) extracts were tested by applying on the larva. The sub lethal concentration of NPV (0.75×10^9) was prepared and ten microlitre of the viral suspension was uniformly smeared to the diet with a blunt end of polished glass rod and the larvae were transferred to the diet for feeding. Three replications were maintained for each treatment. Observations on mortality were taken at 24 hours interval.

3.9. DETERMINATION OF SYNERGISM BETWEEN SOLVENT EXTRACTS OF *V. NEGUNDO* AND ENTOMOPATHOGENS

Based on the larval mortality data generated from the experiments conducted, different types of synergism between *V. negundo* solvent extracts and entomopathogens were assessed using the method of Benz (1971).

3.9.1. Independent Synergism

Independent synergism is a system of two components acting independently and not interfering with each other. If P_M is the probability of death due to microorganisms taken alone, and P_I, the corresponding value for botanicals, the probability of death by combined action is

$$P_{M+I} = P_M + (1- P_I)$$

On the other hand, if the corresponding value of mortality in percentage are used,

$$M_{M+b} = M_M + M_b (1- M_M/100)$$

Where,

M_M = Per cent mortality due to micro organism

M_b = Per cent mortality due to botanicals

M_{M+b} = Per cent combined mortality

3.9.2. Sub additive synergism

Sub additive synergism is a system of two components, which together produce an effect greater than the independent synergism, but less than the algebraic sum of the two single effects. A weak potentiating effect is necessary to produce such a result.

3.9.3. Supplimentary synergism

Supplimentary synergism is a system of two effective components, which together produce an effect greater than the algebraic sum of the single effect ($M_{M+b} > M_M + M_b$).

3.9.4. Potentiating synergism

Potentiating synergism is a system of component A causing the effect M_A (mortality due to component A) and a synergist S which alone causes no effect (Mortality due to synergist S, $M_S=0$), but in combination produce an effect which is significantly greater than M_A . This type of synergism may be found when sub lethal concentration of an insecticide is combined with a microorganism.

3.9.5. Temporal synergism

Temporal synergism is a system, which occurs when two components together kill insects quicker than either component alone.

3.9.6. Economic synergism

Economic synergism is a system of two components which together reduce the damage more than each component alone. Two types included under this are inter specific and intra specific economical synergism.

3.9.7. Synergestic coefficient

It is an index of multiplicative effects. Synergestic coefficient is calculated by the formula:

$Y = x + y + \lambda xy$

$Y = M_M + M_b$ ($M_M + M_b$ = mortality of microorganisms and botanicals)

$x = M_M$ (Mortality due to micro organism alone)

$y = M_b$ (Mortality due to botanicals alone)

$\lambda =$ Synergestic coefficient

If $\lambda = 1$, it is perfect synergism

If $\lambda \geq 1$, it is higher order synergism

If $\lambda \leq 2 \geq 1$, it is lower order synergism

If $\lambda \leq 0$, it is sub multiplicative synergism

3.10. STATISTICAL ANALYSIS

Statistical analysis was done using analysis of variance technique (Panse and Sukhatme, 1985). MSTAT C, SPSS and MS-Excel soft wares were used for computation and analysis.

Results

4. RESULTS

The results of the present study on "Biopotency of Indian Privet, *Vitex negundo* Linn. (Verbenaceae) against *Spodoptera litura* (Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae)" conducted during the period 2005-2008 in the Department of Agricultural Entomology are presented below.

4.1. BIOEFFICACY OF DIFFERENT PLANT PARTS AND SOLVENT EXTRACTS OF V*ITEX NEGUNDO* AGAINST *SPODOPTERA LITURA* AND *HENOSEPILACHNA VIGINTIOCTOPUNCTATA*

Extracts from different parts (leaves, flowers and shoots) were prepared at four concentrations (1, 2, 4 and 6%) of four solvents (hexane, acetone, water and methanol) and tested for their bioefficacy on *S. litura* and *H. vigintioctopunctata*. The results are presented in Tables1 and 2.

4.1.1. Bioefficacy of different parts and solvent extracts of *V. negundo* against *S. litura*

Among the different parts of *V. negundo*, leaf extracts showed significantly highest mortality (37.5 %) followed by shoot (3.54%) and flower (3.54%) on *S. litura* larvae (Fig.1). The mortality of larvae treated with leaf extract ranged from 6.70 to 69.20 per cent. But flower and shoot extracts caused mortality from 1.67 to 5.83 and 0.83 to 5.00 per cent respectively in different concentrations of solvent extracts (Table 1).

Hexane, acetone, water and methanol extracts of leaf with @ 1,2,4 and 6 per cent caused 10 to 80, 6.67 to70, 3.33 to 40 and 6.67 to 86.7 per cent mortality respectively of *S. litura*. The mortality was found to increase with increase in concentration of extracts. Significantly highest mortality (86.00 %) was observed in methanol extract at six per cent concentration and was on par with hexane extracts at six per cent (80.00%). The solvent extracts of acetone at six per cent level also recorded higher mortality of 70.00 per cent, while water

Table 1. Bioefficacy of different solvent extracts from different parts of *Vitex negundo* on *S. litura*

Plant Parts	Treatment concentration (%)	Mortality(%)				
		Hexane extract	Acetone extract	Water extract	Methanol extract	Mean
Leaf	1	*10.000 (0.322) def	6.667 (0.268) ef	3.333 (0.213) ef	6.667 (0.261) ef	6.667 (0.266) bc
	2	30.000 (0.542) cd	26.667 (0.508) cde	6.667 (0.268) ef	43.333 (0.718) c	26.667 (0.509) b
	4	33.333 (0.598) c	50.000 (0.785) bc	33.333 (0.615) c	73.333 (1.042) ab	47.500 (0.760) a
	6	80.000 (1.31) a	70.000 (0.995) ab	40.000 (0.683)c	86.667 (1.209) a	69.167 (1.004)a
Flower	1	3.333 (0.159)f	6.667 (0.261) ef	3.333 (0.213) ef	10.000 (0.322) def	5.833 (0.239)c
	2	3.333 (0.213)ef	0.000 (0.159) f	3.333 (0.213)ef	0.000 (0.159)f	3.333 (0.186) c
	4	3.333 (0.213)ef	0.000 (0.159) f	0.000 (0.159) f	3.333 (0.213) ef	1.667 (0.186)c
	6	0.000 (0.159) f	6.667 (0.261) ef	0.000 (0.159) f	6.667 (0.261) ef	3.333 (0.210)c
Shoot	1	0.000 (0.159) f	6.667 (0.261) ef	6.667 (0.261) ef	3.333 (0.213) ef	4.167 (0.223) c
	2	0.000 (0.159) f	3.333 (0.213) ef	0.000 (0.159) f	0.000 (0.159) f	0.833 (0.173) c
	4	3.333 (0.213)ef	0.000 (0.159) f	6.667 (0.268) ef	6.667 (0.268) ef	4.167 (0.227) c
	6	6.667 (0.261) ef	6.667 (0.261) ef	0.000 (0.159) f	6.667 (0.261) ef	5.000 (0.235) c
Mean		14.444 (0.344)a	15.278 (0.357) a	8.611 (0.281) a	21.111 (0.424) a	

* In a column the means followed by the same letter do not differ significantly.
Figures in paranthesis are arcsine transformed values

Table 2. Bioefficacy of different solvent extracts from different parts of *V. negundo* on *H. vigintioctopunctata*

Plant Parts	Treatment concentration (%)	Mortality(%)				
		Hexane extract	Acetone extract	Water extract	Methanol extract	Mean
Leaf	1	*16.667 (0.408) fghij	16.667 (0.408) fghij	13.333 (0.369)fghij	6.667 (0.268) ij	13.333 (0.363) cd
	2	36.667 (0.650) cdef	43.333 (0.718) cde	26.667 (0.529) efghi	30.000 (0.529) efghi	28.333 (0.539) bc
	4	43.333 (0.717) cde	56.667 (0.852) bcd	30.000 (0.576) defgh	33.333 (0.609) defg	40.833 (0.689) b
	6	86.667 (1.209) a	73.333 (1.033) ab	60.000 (0.893) bc	73.333 (1.030) ab	73.333 (1.041) a
Flower	1	6.667 (0.268) ij	0.000 (0.159) j	3.333 (0.213) j	10.000 (0.299)hij	5.000 (0.235) d
	2	13.333 (0.369)fghij	0.000 (0.159) j	16.667 (0.401) fghij	3.333 (0.213) j	8.333 (0.286) cd
	4	3.333 (0.213) j	6.667 (0.261) ij	3.333 (0.213)j	0.000 (0.159)j	3.333 (0.211) d
	6	6.667 (0.261) ij	13.333 (0.362)fghij	6.667 (0.261) ij	6.667 (0.261) ij	8.333 (0.286) cd
Shoot	1	10.000 (0.299)hij	6.667 (0.261) ij	3.333 (0.213) j	6.667 (0.261) ij	6.667 (0.259) d
	2	13.333 (0.334)hij	0.000 (0.159) j	13.333 (0.334)ghij	10.000 (0.299)hij	9.167 (0.282)cd
	4	3.333 (0.213)j	0.000 (0.159) j	3.333 (0.213)j	6.667 (0.268) ij	3.333 (0.213)d
	6	6.667 (0.261) ij	6.667 (0.261) ij	3.333 (0.213)j	3.333 (0.213)j	5.000 (0.237) d
Mean		20.556 (0.434)a	18.611 (0.399) a	15.278 (0.369)a	13.889 (0.345) a	

* In a column the means followed by the same letter donot differ significantly.
Figures in paranthesis are arcsine transformed values

extracts at the same level recorded only 40.00 per cent mortality (Table1and Fig.2).

4.1.2. Bioefficacy of different parts and solvent extracts of *V. negundo* against *H. vigintioctopunctata*

Among the different plant parts tested, leaf extracts recorded significantly higher mortalities ranged from 13.33 to 73.33 per cent where as flower and shoot extracts caused lowest mortality ranging from 3.33 to 8.33 and 3.33 to 9.17 per cent respectively. Leaf extracts with hexane six per cent caused highest mortality (86.67 %) and lowest with water (60.00%) at six per cent concentration. Acetone and methanol extracts caused 16.67 to 73.33 and 6.67 to 73.33 per cent mortality respectively at different concentrations and they were on par at six per cent concentration.

4.2. MODE OF ACTION OF DIFFERENT EXTRACTS OF *VITEX NEGUNDO*

4.2.1. Ovipositional deterrent action

4.2.1.1. Ovipositional deterrent action of different solvent extracts of V. negundo against S. litura

All the four solvent extracts of *V. negundo* showed significant ovipositional deterrent effect on *S. litura* as compared to control. Lowest number of eggs (22.33) was observed with methanol extract and was significantly superior to all other treatments showing 94.02 per cent reduction in egg laying over control (Tables 3 and 3a). Next to methanol, water extract (6%)was found to reduce egg laying by 85.15 per cent. Eggs laid in hexane and acetone extract were found to be on par by causing 80 per cent reduction in eggs.

In the control treatments (solvents alone), water and methanol showed least inhibition with highest number of eggs (444.30 and 373.67) and were on par with each other while lowest number of eggs were observed in hexane (258) and acetone (289) which were on par with each other (Table 3).

4.2.1.2. Ovipositional deterrent action of different solvent extracts of V. negundo against H. vigintioctopunctata

All concentrations of methanol extracts of *V. negundo* exhibited highest deterrent effect for oviposition of *H. vigintioctopunctata* (Table 3). Cent per cent inhibition of egg laying was observed by methanol extract (6%) (Table 3a). However, methanol extract at 2, 4 and 6 per cent concentrations were on par with each other. Hexane extract at four per cent concentration showed equal ovipositioal deterent action with all concentrations except one percent of methanol extracts. There was no significant difference in the ovipositional deterrency between extracts of acetone (4%), water (2%) and methanol (1%). Acetone extracts showed least deterrent effect for oviposition of *H. vigintioctopunctata* (20-32 eggs). In control, water (38.60 eggs) showed least deterrency which was highest in hexane (29.50 eggs).

4.2.2. Ovicidal action

Ovicidal action of different solvent extracts of *V. negundo* was determined as per the method described in the section 3.4.1 and the results are presented in Tables 4 and 4a.

4.2.2.1. Ovicidal action of different solvent extracts of V. negundo aginst S. litura

Treatment of eggs of *S. litura* with hexane, acetone, water and methanol extracts of *V. negundo* resulted in 46.43 to 91.91, 43.72 to 94.13, 73 to 95.71 and 37.27 to 92.31 per cent hatching respectively (Table 4). Hatching of eggs was found to decrease with increase in concentration of the extract from 1 to 6 per cent. Ovicidal action of one per cent hexane, acetone and water extracts were on par with respective control indicating no effect of *V. negundo* at one per cent concentration. Methanol extract at six per cent was significantly most effective as an ovicidal agent with lowest hatching (37.27 %) and showing 61.34 per cent reduction in hatching over control (Table 4a). Among the solvents (control), hexane showed highest ovicidal action while methanol caused lowest action on

Table 3. Ovipositional deterrent action of different solvent extracts of *V. negundo* against *S. litura* and *H. vigintioctopunctata*

Test Insects	Treatment (%)	*Number of eggs laid (No.) n= 1 pair			
		Hexane extract	Acetone extract	Water extract	Methanol extract
S. litura	1	**200.000(14.114)^{bcd}	179.000(13.131)^{bcde}	288.000(16.949)^{abc}	147.000(11.863)^{cde}
	2	156.000(12.441)^{bcde}	81.667(8.968)^{def}	169.000(12.902)^{bcde}	150.667(11.954)^{cde}
	4	69.333(6.933)^{def}	92.000(7.528)^{def}	95.333(9.721)^{def}	55.333(6.158)^{ef}
	6	51.667(6.061)^{ef}	56.000(6.277)^{ef}	66.000(7.917)^{def}	22.333(3.210)^{f}
	Control	258 (16.07)^{abc}	289 (17.015)^{abc}	444.333(20.969)^{a}	373.667(19.262)^{a}
H. vigintioctopunctata	1	16.333(4.091)^{cdef}	32.000(5.621)^{abc}	28.000(5.288)^{abcd}	23.000(4.796)^{abcde}
	2	12.000(3.086)^{fg}	22.333(4.755)^{abcdef}	23.000(4.838)^{abcde}	1.333(1.178)^{h}
	4	0.667(0.998)^{h}	23.667(4.885)^{abcde}	12.333(3.431)^{ef}	2.000(1.321)^{h}
	6	3.333(1.793)^{gh}	20.000(4.486)^{bcdef}	14.000(3.783)^{def}	0.000(0.707)^{h}
	Control	29.500(5.477)abcd	34.520(5.918)abc	38.667(6.255)a	36.000(6.001)ab

* Mean of ten replications

** Figures in parathesis denote square root transformation. In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

Table. 3a. Ovipositional inhibition of *S. litura* and *H. vigintioctopunctata* as influenced by different solvent extracts of *V. negundo* over control

Test insects	Treatment (%)	*% inhibition of egg laying over control			
		Hexane extract	Acetone extract	Water extract	Methanol extract
Spodoptera litura	1	22.48	38.06	35.18	60.67
	2	39.53	71.74	61.97	59.68
	4	73.13	68.17	78.55	85.19
	6	79.97	80.62	85.15	94.02
Henosepilachana vigintioctopunctata	1	44.63	7.30	27.59	36.11
	2	59.32	35.30	40.52	96.29
	4	97.74	31.44	68.10	94.44
	6	88.70	42.06	63.79	100

Table 4. Ovicidal action of different solvent extracts of *V. negundo* against *S. litura* and *H. vigintioctopunctata*

Test insects	Treatment (%)	*Hatching of eggs (%)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
Spodoptera litura	1	89.71^{abc}	93.95^{ab}	95.71^{ab}	92.31^{ab}
	2	91.91^{ab}	94.13^{ab}	83.29^{abcd}	75.01^{cd}
	4	56.42^{e}	43.72^{ef}	78.66^{bcd}	52.90^{ef}
	6	46.43^{ef}	44.66^{ef}	73.00^{d}	37.27^{f}
	Control	89.3^{abc}	92.5^{ab}	93.98^{ab}	96.40^{a}
Henosepilachna. vigintioctopunctata	1	73.500^{a}	19.440^{b}	9.247^{b}	71.76^{a}
	2	38.200^{b}	18.070^{b}	3.617^{bc}	56.15^{b}
	4	9.870^{c}	0.000^{c}	0.000^{c}	42.06^{c}
	6	4.783^{c}	0.000^{c}	0.000^{c}	38.47^{c}
	Control	74.730^{a}	74.160^{a}	74.810^{a}	76.580^{a}

* Mean of ten replications
** In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

Table 4a. Inhibition of hatching of *S. litura* and *H. vigintioctopunctata* as influenced by different solvent extracts of *V. negundo*

Insects	Treatment (%)	*Inhibition of hatching of eggs over control (%),			
		Hexane extract	Acetone extract	Water extract	Methanol extract
Spodoptera litura	1	-0.46	-1.56	-1.84	4.24
	2	-2.92	-1.76	11.37	22.19
	4	36.82	52.74	16.30	45.12
	6	48	51.72	22.32	61.34
Henosepilachna. vigintioctopunctata	1	1.65	73.71	87.64	6.29
	2	48.88	75.63	95.17	26.68
	4	86.79	100	100	45.07
	6	93.60	100	100	49.76

egg hatching. Acetone and water caused no significant difference on their effect on hatching.

4.2.2.2. Ovicidal action of different solvent extracts of V. negundo aginst H. vigintioctopunctata

Significant ovicidal action was observed with hexane, acetone, water and methanol extracts (4 and 6%) of *V. negundo*. Cent per cent hatching inhibition was observed in acetone and water extracts at four and six per cent concentrations (Table 4a). There was no significant effect on egg hatching between the four solvents (hexane, acetone, water and methanol). However, extracts with hexane and methanol (1%) were on par with their respective controls indicating no ovicidal action at their lowest concentration of one per cent. Hexane extract at six per cent also prevented egg hatching with 93.60 per cent inhibition over control. Water extracts at one per cent was found to be on par with four per cent extracts of hexane, acetone and methanol (Table 4).

4.2.3. Growth and developmental effects

4.2.3.1. Effect of different solvent extracts of V. negundo on pupal weight of S. litura on castor leaf, semi synthetic diet and banana leaf

The results of the effect of different solvent extracts of *V. negundo* on the pupal weight of *S. litura* reared on castor, semi-synthetic diet and banana are given in Tables 5 and 5a. The increase in concentration from one to six per cent hexane, acetone water and methanol extracts of *V. negundo* was found to reduce the weight of pupae from 0.217 to 0.117g, 0.203 to 0.115g, 0.193 to 0.117g and 0.197 to 0.130g respectively when *S. litura* was reared on castor. There was no significant difference in the pupal weight among the extracts at six per cent. Methanol at two per cent was also found to be on par with all the solvent extracts at six per cent (Table 5). However acetone extract caused a highest reduction in pupal weight (55.25 %) (Table 5a). Control (solvents alone) of all the solvent extracts showed a significantly higher pupal weights indicating the effects of *V. negundo* extracts.

Table 5. Effect of solvent extracts of *V. negundo* on pupal weight of *S. litura* reared on castor, semi-synthetic diet and banana

Hosts	Concentration %	Pupal weight (g)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
**Castor leaf	1	*0.217^{bc}	0.203^{bcd}	0.193^{cd}	0.197^{cd}
	2	0.173^{de}	0.153^{ef}	0.170^{de}	0.123^{fgh}
	4	0.147^{efg}	0.154^{ef}	0.140^{efgh}	0.107^{h}
	6	0.117^{fgh}	0.115^{gh}	0.117^{fgh}	0.130^{fgh}
	Control	0.230^{abc}	0.257^{a}	0.220^{bc}	0.237^{ac}
Semi-synthetic diet	1	0.427^{bcd}	0.217^{g}	0.473^{ab}	0.203^{gh}
	2	0.317^{f}	0.173^{ghij}	0.343^{ef}	0.183^{ghi}
	4	0.323^{f}	0.147^{hij}	0.353^{ef}	0.183^{ghi}
	6	0.173^{ghij}	0.117^{j}	0.197^{gh}	0.137^{ij}
	Control	0.410^{cd}	0.390^{de}	0.453^{abc}	0.500^{a}
Banana leaf	1	0.190^{a}	0.182^{ab}	0.200^{a}	0.176^{ab}
	2	0.192^{a}	0.185^{ab}	0.193^{a}	0.182^{ab}
	4	0.134c	0.154^{bc}	0.141^{c}	0.121^{c}
	6	0.122^{c}	0.129^{c}	0.143^{c}	0.070^{d}
	Control	0.203^{a}	0.197^{a}	0.193^{a}	0.203^{a}

*In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

** Separate CRD analysis was done for castor, semi-synthetic diet and banana

Table 5a. Reduction of pupal weight of *S. litura* as influenced by different solvent extracts of *V. negundo* over control

Solvent extracts (6% concentration)	Pupal weight reduction over control (%)		
	Castor	Semi-synthetic diet	Banana
Hexane	49.13	57.80	39.90
Acetone	55.25	70	34.52
Water	46.82	56.51	25.91
Methanol	45.15	72.60	65.52

When the larvae were reared on semi-synthetic diet, significantly lowest pupal weight (0.117 g) was observed with acetone extract (6%). At six per cent concentration, water extract resulted in highest pupal weight (0.197g) . In control also, same trend of lowest pupal weight (0.390g) in acetone and highest pupal weight in methanol (0.500g) was observed. Among the solvents (control), acetone caused lowest weight and methanol caused highest weight, but solvent extracts of both acetone and methanol caused highest reduction in pupal weight thus indicating the higher potency of *V. negundo* on pupal development when reared on semi-synthetic diet.

The same trend of decrease in pupal weight with the increase in concentrations of *V. negundo* extracts was recorded in *S. litura* reared on banana leaf also. Methanol extract recorded significantly lowest pupal weight (0.07g) while highest pupal weight (0.143g) was observed with aqueous extract. However extract with hexane at four and six per cent, acetone at six per cent and water at four and six per cent were on par (Table 5).

Among all the hosts, highest reduction in pupal weight was on semi-synthetic diet reared *S. litura*. Methanol extract caused highest reduction in pupal weight (45.15 to 72.60 %) followed by acetone extract (34.52 to 70 %) and lowest reduction in pupal weight was observed in water extract (25.91 to 56.51 %) (Table 5a).

4.2.3.2. Effect of different solvent extracts of V. negundo on percentage pupation of S. litura on castor, semi synthetic diet and banana

The results are presented in Tables 6 and 6a.

All the concentrations of the four solvent extracts of *V. negundo* caused significant reduction in the pupation of *S. litura* reared on castor and banana leaves.

On castor, six per cent hexane and acetone extracts caused significantly lowest pupation (3.33% each) showing maximum reduction (96.15 and 96.30 %) over control and was followed by methanol extract with 92.86 per cent reduction

Table 6. Effect of solvent extracts of *V. negundo* on pupation of *S. litura* reared on castor, semi-synthetic diet and banana

Hosts	Concentration %	Percentage pupation (%)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
**Castor leaf	1	*26.667(5.191)^{cdefg}	20.000(4.430)^{efgh}	53.333(7.307)^{bc}	16.667(4.099)^{fgh}
	2	36.667(5.999)^{cdef}	33.333(5.719)^{cdef}	50.000(7.083)^{c}	13.333(3.157)^{ghi}
	4	23.333(4.860)^{defg}	6.667(2.396)^{hij}	46.667(6.802)^{cd}	6.667(2.396)^{hij}
	6	3.333(1.551)^{ij}	3.333(1.551)^{i}	40.000(6.275)^{cde}	6.667(2.396)^{hij}
	Control	86.667(9.323)^{a}	90.000(9.482)^{a}	83.333(9.152)^{ab}	93.333(9.684)^{a}
Semi-synthetic diet	1	86.67^{abcd}	93.33^{ab}	93.33^{ab}	56.67^{f}
	2	80.00^{abcde}	60.00^{ef}	96.67^{a}	60.00^{ef}
	4	73.33^{bcdef}	73.33^{bcdef}	73.33^{bcdef}	23.33^{g}
	6	63.33^{ef}	70.00^{cdef}	66.67^{def}	20.00^{g}
	Control	86.67^{abcd}	96.67^{a}	96.67^{a}	90.00^{abc}
Banana leaf	1	43.33^{de}	26.67^{efgh}	60.00^{bc}	23.33^{fgh}
	2	53.33^{cd}	30.00^{efgh}	70.00^{b}	26.67^{efgh}
	4	40.00^{def}	26.67^{efgh}	36.67^{defg}	23.33^{fgh}
	6	26.67^{efgh}	20.00^{gh}	40.00^{def}	13.33^{h}
	Control	96.67^{a}	93.33^{a}	90^{a}	93.33^{a}

* Figures in parathesis denote square root transformation. In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

** Separate CRD analysis was done for castor, semi-synthetic diet and banana

Table 6a. Reduction of pupation of *S. litura* as influenced by different solvent extracts of *V. negundo* over control

Solvent extracts (6% concentration)	Pupal weight reduction over control (%)		
	Castor	Semi-synthetic diet	Banana
Hexane	96.51	26.93	72.41
Acetone	96.30	27.59	78.57
Water	52	31.03	55.56
Methanol	92.86	77.78	85.72

in pupation (Table 6a). water extract at the same concentration caused highest pupation (40%). All control treatments (solvents alone) were on par.

Pupation of *S. litura* reared on semi-synthetic diet was also found to decrease with increase in extract concentration. Methanol extract with four and six per cent caused significantly lowest pupation (23.33 and 20.00%) and were on par. At six per cent concentration highest pupation was observed with acetone extract (70%). In control, hexane recorded lowest pupation (86.67%) and highest pupation was observed in acetone and water (96.67% each) and were on par.

The same trend of reducing pupation with increase in *V. negundo* extracts was observed in banana leaf also. At six per cent concentration, methanol extract caused a lowest pupation of 13.33 per cent and water extract caused a highest pupation (40%). When *S. litura* larvae were reared on banana after treatment with *V. negundo* extracts, pupation rate was significantly reduced in all the solvent extracts of *V. negundo* as compared to control. But the different solvents of hexane, acetone , water and methanol (control) caused no significant difference in pupation of *S. litura*.

Methanol extracts at all the concentrations significantly reduced pupation rate with the lowest being at six per cent concentration (13.3%). The lower concentrations of methanol and acetone extracts reduced the pupation rate (26.67- 20%). Aqueous extracts indicated highest rate of pupation ranging from 40 to 60 % with six and one per cent concentrations respectively.

Results on the influence of *V. negundo* extracts on the reduction in pupation of *S. litura* reared on different hosts is presented in Table 6a. Hexane, acetone and methanol extracts caused highest reduction of 96.15, 96.30 and 92.86 per cent over control respectively while water extract produced only 52 per cent reduction of pupation of *S. litura* reared on castor . *V. negundo* extracts caused lowest reduction of pupation (26.93 to 77.78 %) of *S. litura* reared on semi-synthetic diet. In banana pupation was reduced in the range of 55.56 to 85.72 per cent by water and methanol extract.

4.2.3.3. Effect of different solvent extracts of V. negundo on larval duration of S. litura on castor, semi-synthetic diet and banana

Results on larval duration of *S. litura* reared on castor, semi-synthetic diet and banana treated with different solvent extracts of *V. negundo* are given in Table 7. On castor, the larval duration of *S. litura* varied from 12.33 to 18, 15 to 19, 12 to18 and 11.67 to 15.33 days when treated with hexane, acetone, water and methanol extracts of *V. negundo* respectively. Larval duration was extended to 19 days when leaf extract was treated with six percent acetone extract and reduced to15.33 days in methanol extract. Prolonged larval duration (16.33 – 18 days) was also observed with hexane extracts (4 and 6%) and acetone extract (4 and 2 %) of *V. negundo* (16.67–17 days). Larval duration at two and four per cent concentrations of acetone extracts were found to be on par with each other. The aqueous extract at six per cent concentration only showed significant increase in larval period (18 days). The higher concentrations of four and six per cent of methanol extracts caused significantly longer (15.67 – 15.33 days) larval period. However, both concentrations were on par. No significant difference in larval period of *S. litura* was observed between the solvents (control) of hexane, acetone, water and methanol.

S. litura reared on synthetic diet showed significant increase in larval duration on treatments with *V. negundo* extracts of hexane, acetone, water and methanol. Hexane extracts at one to six per cent resulted in 14.67 to 24 days as against 13.3 days in control (solvents alone). The larval period ranged from 16.67 to 26 days, 10.67 to 26.33 days and 15 to 19 days in acetone, methanol and aqueous extracts respectively as compared to 14, 13 and 13 days in control treatments. Control treatments of hexane and acetone and water and methanol were on par.

In banana, aqueous extract of *V. negundo* at six per cent caused longest larval duration (17.67 days) and hexane extract at one per cent produced lowest larval period of 12.67 days in *S. litura*. No significant difference in the larval period was observed between hexane extract (4%), acetone extract (1%),

Table 7. Effect of solvent extracts of *V. negundo* on larval duration of *S. litura* reared on castor, semi-synthetic diet and banana

Hosts	Concentration %	Larval duration (days)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
**Castor leaf	1	*12.33^{cd}	15.00^{ab}	12.00^{d}	11.67^{d}
	2	12.00^{d}	17.00^{ab}	12.00^{d}	12.00^{d}
	4	16.33^{ab}	16.67^{ab}	12.00^{d}	15.67^{b}
	6	18.00^{abc}	19.00^{d}	18.00^{ab}	15.33^{b}
	Control	11.67 ^{d}	11.67 ^{d}	11.33 ^{d}	11.33^{d}
Semi-synthetic diet	1	14.67^{fgh}	17.33^{cde}	15.00^{efgh}	15.67^{defg}
	2	15.67^{defg}	16.67^{cdef}	15.33^{efgh}	10.67^{i}
	4	19.00^{c}	21.67^{b}	17.33^{cde}	18.00^{cd}
	6	24.00^{a}	26.00^{a}	19.00^{c}	26.33^{a}
	Control	13.33^{gh}	14.00^{gh}	13.00^{hi}	13.00^{hi}
Banana leaf	1	12.67^{e}	14.67^{bcde}	13.67^{cde}	16.33^{abc}
	2	15.67^{abcd}	13.00^{de}	14.33^{cde}	14.33^{cde}
	4	15.00^{bcde}	17.33^{ab}	15.67^{abcd}	16.00^{abc}
	6	17.33^{ab}	15.33^{abcde}	17.67^{a}	15.00^{bcde}
	Control	14.33^{cde}	14.67^{bcde}	14.33^{cde}	14.00^{cde}

*In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

** Separate CRD analysis was done for castor, semi-synthetic diet and banana

Table 7a. Increase in larval period of *S. litura* as influenced by different solvent extracts over control

Solvent extracts (6% concentration)	Increase in larval period over control (%)		
	Castor	Semi-synthetic diet	Banana
Hexane	54.24	80.04	20.94
Acetone	62.81	85.71	4.49
Water	58.87	46.15	23.31
Methanol	35.30	102.54	7.14

methanol extract (6%) and control treatment with acetone only which was significantly higher than the other solvent treatments (controls).

A maximum larval duration of 26.33 days with highest (102.54 %) increase in duration over control was recorded by methanol extract (6%) on synthetic diet. Prolonged larval duration was also observed in hexane and acetone extract (80.04 and 85.71 %) on semi-synthetic diet (Table 7a). In control treatment, the larval period was highest in banana and lowest in castor.

4.2.4. Antifeedant action

Feeding deterrent action of different solvent extracts were analyzed and the results are given in Tables 8 to 9.

4.2.4.1. Effect of different solvent extracts of V. negundo on the percentage leaf protection against S. litura on castor, synthetic diet and banana

Solvent extracts of *V. negundo* were tested for their antifeedant action and the results are given in Table 8.

All the treatments recorded very low leaf protection values indicating that *V. negundo* extracts showed no antifeedant effect against *S. litura*.

In case of castor, maximum leaf protection of 22.619 per cent was recorded by water extract at six per cent concentration and it was followed by methanol and hexane extracts at one per cent concentration (19.048 and 18.651 %). None of the treatments (except water extract 6%) recorded more than 20 per cent leaf protection. Lowest leaf protection (-15.079 %) was recorded by the acetone extracts at four per cent concentration and was immediately followed by water extracts at one per cent level (-13.095 %), methanol extracts at six per cent (-5.357 %), water extract at two per cent (-5.159 %), acetone extracts at six per cent (-5.159 %) and hexane extracts at two per cent (-1.190 %) also recorded very low leaf protection. All these treatments recorded negative values indicating that consumption was more in treatments than in control.

The same trend was also observed in case of *S. litura* larvae reared on semi-synthetic diet also. In this case only water extracts at four per cent

Table 8. Effect of solvent extracts of *V. negundo* on percentage leaf protection of *S. litura* reared on castor, semi-synthetic diet and banana

Hosts	Treatment concentration %	Host protection (%)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
Castor leaf	1	18.651	12.698	-13.095	19.048
	2	-1.190	14.683	-5.159	7.143
	4	14.683	-15.079	2.778	14.087
	6	8.730	-5.159	22.619	-5.357
Semi-synthetic diet	1	-23.11	5.068	15.428	-10.360
	2	-10.698	12.162	-50.113	-21.622
	4	0.450	5.293	25.563	-40.766
	6	-60.473	-22.196	5.180	15.541
Banana leaf	1	-34.104	5.395	-29.480	-18.015
	2	-57.996	21.773	-10.405	-42.004
	4	-19.171	7.129	36.609	30.539
	6	0.867	42.389	41.137	39.499

Table 9. Effect of *V. negundo* solvent extracts on the percentage of leaf protection against *H. vigintioctopunctata*

Concentration(%)	Leaf protection (%)			
	Hexane extract	Acetone extract	Water extract	Methanol extract
1	-18.519^{cdef}	16.667 ^{ab}	-33.148 ^{efg}	-10.926 ^{bcde}
2	-36.111 ^{g}	-6.481 ^{abcd}	-33.704 ^{fg}	- 7.778^{bcd}
4	-19.815 ^{cdef}	15.926 ^{ab}	-23.519 ^{cdefg}	3.519^{abc}
6	-20.741 ^{abcd}	22.963 ^{a}	-25.741 ^{defg}	-7.037 ^{abcd}

concentration recorded 25 per cent leaf protection (25.563 %). Most of the treatments recorded negative values indicating that consumption was more in treatments than in control. Methanol extracts at one, two and four per cent (-10.36, -21.622 and –40.766 %), water extracts at two per cent (-50.113 %), acetone extracts at six per cent (-22.196 %) and hexane extracts at one, two and six per cent (-23.311, -10.698, and –60.473 %) recorded very low leaf protection values indicating that these extracts had no antifeedant property and consumption was more in treatments than in control.

Similar were the results when the larvae were reared on banana leaves. All the solvent extracts of *V. negundo* except acetone extracts recorded negative leaf protection at lower concentrations indicating that these extracts were inferior as antifeedants. Hexane extracts at one, two and four per cent (-34.014, -57.996 and –19.171 %), methanol extracts at one and two per cent (-18.015 and –42.004 %) concentrations recorded negative leaf protection values indicating that consumption was more in these treatments than in control and there was no feeding deterrency for these extracts. More than 25 per cent leaf protection was recorded by acetone extracts at six per cent (42.389 %), water extracts at six and four per cent (41.137 and 36.609 %) and methanol extracts at six and four per cent (39.499 and 30.539 %) concentrations respectively. But none of these treatments recorded more than 50 per cent leaf protection.

4.2.4.2. Effect of different solvent extracts of V. negundo on the percentage leaf protection against H. vigintioctopunctata

Hexane and water extracts at one, two, four and six per cent concentrations resulted in negative leaf protection values indicating that consumption was more in theses treatments than in control and there was no feeding deterrency for *V. negundo* extracts. Methanol extract at all the concentrations except at four per cent also resulted in negative leaf protection values (-10.926, -7.778 and –7.037 %) (Table 9).

None of the treatments recorded more than 25 per cent leaf protection. Lowest leaf protection and highest consumption was observed in treatment with hexane extract at two per cent concentration and was immediately followed by water extract at one and two per cent (-36.111,–33.148 and –33.704%) concentrations. Highest leaf protection was observed in acetone extract at six per cent (22.963 %) and was followed by the same extract at one and four per cent (16.667 and 15.926 %) concentrations respectively (Table 9).

4.2.5. Larval starvation

Larval starvation was worked out in different treatments and the results are presented in Tables 10 and 11.

4.2.5.1. Effect of different solvent extracts of V. negundo on larval starvation against S. litura on castor leaf, semi-synthetic diet and banana leaf

Results of the study on percentage larval starvation are presented in Table 10**.**

When the treated larvae of *S. litura* were reared on castor, highest larval starvation was recorded by the acetone extracts at six per cent (51.482%) followed by methanol extract at six per cent (49.630%). Lowest larval starvation was recorded by water extracts at one and two per cent (39.259 and 39.815%). Larval starvation increased with increase in concentration of methanol extract (40 to 49 %) and hexane extracts (44 to 49.61 %) from one to six per cent.

Larvae when reared on semi-synthetic diet, comparatively lower starvation was observed. Larval starvation values ranged between 14.403 and 22.840 per cent in hexane extracts to 14.503 and 31.070 per cent for acetone extracts, 19.074 to 24.486 per cent for water extracts and 24.074 to 44.239 per cent for methanol extracts. Highest larval starvation was recorded by six per cent methanol extract (44.239 %) and lowest by hexane extracts at one per cent level (14.403 %).

Larval starvation showed an increasing trend from one to six per cent concentrations except at four per cent concentration of acetone extract in case of *S. litura* larvae reared on banana. *V. negundo* extracts of methanol at six per cent

Table10. Effect of *V. negundo* solvent extracts on larval starvation of *S. litura* on castor, semi-synthetic diet and banana

Hosts	Treatment Concentration %	Larval starvation (%)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
Castor leaf	1	44.444	44.815	39.259	40.185
	2	45.370	47.592	39.815	43.148
	4	48.148	41.111	45.926	47.407
	6	49.630	51.482	45.000	49.445
Semi-synthetic diet	1	14.403	27.572	20.782	24.074
	2	17.078	14.403	19.074	29.218
	4	18.107	20.370	32.510	37.654
	6	22.840	31.070	24.486	44.239
Banana leaf	1	17.190	14.350	-2.541	26.457
	2	28.251	49.327	30.643	28.999
	4	36.771	43.797	55.904	68.909
	6	52.765	54.559	72.945	74.290

Table 11. Effect of *V. negundo* solvent extracts on larval starvation of *H. vigintioctopunctata*

Concentration(%)	Larval starvation (%)			
	Hexane extract	Acetone extract	Water extract	Methanol extract
1	30.769^{def}	69.231^{abc}	47.008^{cde}	18.803^{ef}
2	46.154^{cde}	70.085^{abc}	41.880^{cdef}	17.949^{f}
4	45.299^{cde}	78.632^{abc}	68.376^{bc}	64.102^{bcd}
6	117.094^{a}	77.778^{abc}	66.667^{bcd}	101.709^{ab}

level recorded highest larval starvation (74.290%). Water extracts at the same level also recorded higher larval starvation of 72.945 per cent. Water extracts at one per cent concentration recorded a negative value of –2.541 per cent. Acetone and hexane extracts also recorded lower larval starvation (14.350 and 17.190 %) at one per cent level.

4.2.5.2. Effect of different solvent extracts of V. negundo on the percentage larval starvation against H. vigintioctopunctata

Results on the effect of *V. negundo* solvent extracts on larval starvation of *H. vigintioctopunctata* are presented in Table 11.

Hexane extracts at six per cent level recorded highest larval starvation (117.094%) followed by six per cent methanol extract. All concentrations of acetone extract (1, 2, 4 and 6%) recorded more than 50 per cent larval starvation with the highest at four per cent level (78.632 %). Larval starvation ranged from 41.88 to 68.34 per cent in water extract. At six per cent level water extract caused lowest larval starvation (66.667 %). Methanol extract also resulted in higher larval starvation (101.709) at six per cent concentration.

4.2.6. Approximate Digestibility (AD)

Effect of different solvent extracts of *V. negundo* on AD of *S. litura* and *H. vigintioctopunctata* were worked out as per 3.4.6 and the results are presented in Tables 12 and 13.

4.2.6.1. Effect of different solvent extracts of V. negundo on the Approximate Digestibility (AD) of S. litura on castor, semi-synthetic diet and banana

All the four solvent extracts of *V. negundo* caused significantly higher AD on *S. litura* reared on castor than their respective control treatment (solvents alone). Highest AD was observed at four per cent hexane extract (96.52 %) and two and six per cent acetone extracts (96.10 and 96.12 %) and they were on par. Methanol extracts showed lowest AD values ranged from 61.53 to 86.06 per cent (Table 12).

Table 12. Effect of different solvents on approximate digestibility (AD) of *S. litura* on castor, semi-synthetic diet and banana

Hosts	Concentration %	Approximate digestibility (%)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
**Castor leaf	1	*94.72^{ab}	93.75^{ab}	93.70^{ab}	61.53^{cd}
	2	90.61^{ab}	96.10^{a}	85.28^{ab}	76.37^{bc}
	4	96.52^{a}	93.39^{ab}	93.22^{ab}	82.72^{ab}
	6	92.82^{ab}	96.12^{a}	93.16^{ab}	86.06^{ab}
	Control	50.51^{d}	47.09^{d}	47.95^{d}	55.16^{d}
Semi-synthetic diet	1	90.475^{abcd}	95.279^{ab}	91.038^{abc}	87.419^{abcde}
	2	91.604^{abc}	92.633^{ab}	93.539^{ab}	92.407^{ab}
	4	89.271^{abcd}	93.623^{ab}	91.976^{ab}	90.467^{abcd}
	6	94.074^{ab}	96.557^{a}	84.018^{abcde}	92.402^{ab}
	Control	68.458^{cde}	69.408^{e}	77.010^{de}	69.894^{bcde}
Banana leaf	1	43.23^{ab}	74.19^{a}	85.62^{a}	83.88^{a}
	2	53.81^{ab}	54.64^{ab}	72.14^{a}	67.48^{ab}
	4	61.71^{a}	76.011^{a}	75.91^{a}	63.06^{abc}
	6	28.72^{b}	74.79^{a}	65.46^{ab}	77.59^{a}
	Control	88.878^{a}	85.621^{a}	76.288^{a}	68.781^{a}

* In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

** Separate CRD analysis was done for castor, semi-synthetic diet and banana

Table 13. Effect of different solvent extracts of *V. negundo* on AD of *H. vigintioctopunctata*

Concentration	Approximate digestibility (%)			
	Hexane extract	Acetone extract	Water extract	Methanol extract
1	99.89^{a}	99.12^{abc}	98.47^{bc}	98.49^{bc}
2	99.16^{ab}	99.09^{abc}	99.16^{ab}	98.46^{bc}
4	98.93^{abc}	98.89^{bc}	98.51^{bc}	98.67^{abc}
6	99.40^{a}	98.33^{c}	98.82^{abc}	98.57^{bc}
Control	97.108^{d}	98.084^{cd}	98.138^{cd}	97.930^{d}

In semi-synthetic diet also *S. litura*, recorded significantly higher AD values with highest AD of 96.557 per cent recorded by acetone extracts at six per cent concentration. AD varied from 87.419 to 92.407 per cent in methanol extract while in aqueous extract, AD was in the range from 84.018 to 93.539 per cent. Hexane extracts resulted in AD from 89.271 to 94.074 per cent. AD in control (solvents alone) ranged from 68.458 per cent in hexane to 77.01 in water.

In banana, significantly higher AD was observed in control of extracts. Hexane at six per cent recorded lowest AD (28.72 %) and highest at two per cent (53.81 %). AD in aqueous extracts ranged from 65.46 per cent (6 %) to 85.62 per cent at one per cent extract. Methanol extracts showed a variation in AD from 63.06 at four per cent extract to 83.88 per cent at one per cent extract. AD of the all the solvents (in control) were on par.

4.2.6.2. Effect of different solvent extracts of V. negundo on the Approximate Digestibility (AD) of H. vigintioctopunctata

All the treatments recorded more than 95 per cent AD (Table 13). Highest AD was observed in hexane extracts at one and six per cent concentrations (99.89 and 99.40 %) and were on par with each other. Water extracts at two per cent also recorded higher AD and was followed by acetone extracts at one per cent (99.16 and 99.12 %) concentrations. *V. negundo* extracts at different concentrations (1, 2, 4 and 6%) recorded higher AD when compared to respective controls. Lowest AD was observed in the control treatments (solvents alone) with methanol, hexane, acetone and water.

4.2.7. Efficiency of conversion of ingested food to body substance (ECI)

ECI of *S. litura* and *H. vigintioctopunctata* in different treatments were calculated and the results are presented in Tables 14 and 15 and Figs. 12 and 13.

4.2.7.1. Effect of different solvent extracts on ECI of S. litura on castor, semi-synthetic diet and banana

The results are presented in Table 14 and 14a.

Table 14. Effect of different solvent extracts of *V. negundo* on Efficiency of Conversion of Ingested food to body substances (ECI) of *S. litura* on castor, semi-synthetic diet and banana

Hosts	Concentration %	ECI (%)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
**Castor leaf	1	*7.353^{def}	6.813^{def}	10.263^{de}	13.343^{cd}
	2	4.833^{efg}	3.393^{efg}	10.123^{de}	7.983^{def}
	4	2.778^{efg}	8.373^{def}	7.643^{def}	3.226^{efg}
	6	0.417^{fg}	-1.310^{g}	4.613^{efg}	0.703^{fg}
	Control	29.57^{bc}	37.83^{a}	26.90^{bcd}	29.60^{b}
Semi-synthetic diet	1	12.536(3.871)^{abcd}	17.682(4.491)^{ab}	15.144(4.257)^{abc}	10.189(3.617)^{bcde}
	2	12.948(3.910)^{abcd}	14.058(4.061)^{abc}	11.864(3.690)^{abcde}	6.882(2.989)^{cdef}
	4	14.426(4.201)^{abc}	9.658(3.403)^{bcdef}	8.585(3.240)^{bcdef}	2.600(2.092)^{fg}
	6	5.429(2.521)^{defg}	3.980(2.276)^{efg}	12.295(3.983)^{abcd}	0.061(1.217)^{g}
	Control	26.060(5.154)^{ab}	33.231(5.808)^{a}	22.504(4.796)^{ab}	22.481(4.794)^{ab}
Banana leaf	1	19.17^{abcd}	33.95^{ab}	30.93^{abc}	12.24^{bcde}
	2	13.31^{bcde}	25.22^{bcde}	16.72^{def}	12.40^{cde}
	4	12.59^{bcde}	10.84^{def}	4.41^{gh}	-9.32^{hij}
	6	4.42^{gh}	4.24^{gh}	-11.60^{h}	-14.81^{i}
	Control	29.932^{b}	30.932^{abc}	51.785^{a}	38.282^{ab}

* Figures in paranthesis denote square root transformation. In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

** Separate CRD analysis was done for castor, semi-synthetic diet and banana

Table 14 a. Reduction in ECI of *S. litura* as influenced by different solvent extracts over control

Solvent extracts (6% concentration)	Reduction in ECI over control (%)		
	Castor	Semi-synthetic diet	Banana
Hexane	98.59	79.17	85.23
Acetone	103.46	88.02	86.29
Water	82.85	45.35	122.40
Methanol	97.63	99.73	138.69

Irrespective of the hosts, there was a declining trend in ECI with increase in concentrations except in *V. negundo* extracts of water six per cent on semi-synthetic diet. The increase in concentrations from one to six per cent of hexane, acetone, water and methanol extracts of *V. negundo* was found to reduce ECI from 7.353 to 0.417, 6.813 to -1.310, 10.263 to 4.613 and 13.343 to 0.703 per cent respectively when *S. litura* was reared on castor. *V. negundo* extracts of hexane and methanol six per cent caused no significant difference in ECI. The lowest ECI of -1.310 per cent was observed in case of acetone extract of *V. negundo* at six per cent concentration. Two per cent concentrations of hexane and acetone extracts recorded lower ECI (4.833 and 3.393 %) and were equally effective with water extracts at six per cent (4.613 %) and extracts of methanol at four per cent (3.226 %) concentrations. Highest ECI of 37.83 % was observed in the control treatment with acetone and lowest of 26.90 per cent in water. All the solvent extracts of *V. negundo* recorded lower ECI when compared to the control (solvents alone) treatments of *S. litura* larvae reared on semi-synthetic diet. ECI was found to decrease with increase in concentration of the extracts from one to six per cent except hexane (2%) and water (6%) extracts. Lowest ECI (0.061 %) was caused by methanol extract at six per cent followed by four per cent concentration (2.600 %).

The ECI in control treatments (solvents alone) of banana recorded a significant variation between solvents. Highest ECI of 51.785 per cent was observed in water and followed by methanol (38.28 %), acetone (30.932 %) and hexane (29.932 %). Among different solvents, hexane recorded a significantly lower ECI of 29.932 per cent..

In banana also, methanol extracts of *V. negundo* at four and six per cent levels recorded very low ECI values of -9.32 and -14.81 per cent respectively. Water extracts at six per cent level (-11.60 %) was also equally effective as methanol extract at four per cent concentration. Water extract at four per cent level also recorded lower ECI (4.41%) and was on par with acetone and hexane extracts at six per cent (4.24 and 4.42 %) levels.

When the larvae were reared on castor, highest reduction in ECI over control was recorded by acetone extract (103.46 %). In case of castor reared larvae, hexane and methanol extracts also resulted in higher reductions in ECI (98.59 and 97.63 %). Methanol extract resulted in highest reduction in ECI when the larvae were reared on both semi-synthetic diet and banana (99.73 and 138.69 %) (Table 14 a).

In case of larvae reared on banana, water extracts of *V. negundo* at six per cent concentration significantly reduced ECI proving its potency as a growth inhibitor. However, its effect on semi-synthetic diet and castor were comparatively lower when compared to acetone and hexane extracts indicating influence of host food material on ECI of *S. litura* larvae.

4.2.7.2. Effect of different solvent extracts of V. negundo on ECI of H. vigintioctopunctata

The data on the influence of *V. negundo* solvent extracts on ECI of *H. vigintioctopunctata* are presented in Table 15.

All the *V. negundo* extracts recorded very low ECI when compared to respective control treatments indicating its effect in reducing ECI of *H. vigintioctopunctata*.

Complete growth inhibition with weight loss was observed in hexane and methanol extracts at six per cent (-7.268 % and –5.20 %) and acetone extracts at four and six per cent (-0.406 and –0.857 %) concentrations respectively. Acetone extracts of *V. neugndo* at one and two per cent concentrations (1.432 and 1.769 %) were on par with solvent extracts of hexane at two and four per cent (4.879 and 5.610 %), water extracts at four and six per cent (1.244 and 1.624 %) and methanol extracts at four per cent (2.745 %) concentrations respectively. All the solvent extracts except methanol extracts at one and two per cent recorded less than 10 per cent ECI at lower concentrations it self (1 %).

Hexane, acetone and methanol extracts of *V. negundo* recorded more than 100 per cent reduction in ECI (120.87, 103.18 and 118.97 % respectively) (Table 15 a).

4.2.8. Efficiency of conversion of digested food to body substances (ECD)

Effect of different solvent extracts of *V. negundo* on ECD of *S. litura* and *H. vigintioctopunctata* are presented in Tables16 and 17.

4.2.8.1. Effect of different solvent extracts of V. negundo on ECD of S. litura on castor, semi-synthetic diet and banana

Results on the effect of *V. negundo* solvent extracts on ECD of *S. litura* larvae reared on castor, semi-synthetic diet and banana are presented in Table 16.

Solvent extracts of *V. negundo* at all the tested concentrations recorded significantly lower ECD when compared to respective control (solvents alone) treatments indicating efficacy of *V. negundo* extracts in reducing ECD of *S. litura* larvae. ECD showed a reducing trend from lower to higher concentrations except in treatment with acetone extract at four per cent. Hexane and methanol extracts at six per cent concentration recorded significantly lowest ECD (0.482 and 0.991 %) and were on par.

All the solvent extracts at six per cent recorded more than 90 per cent reduction in ECD when compared to control and maximum reduction in ECD was recorded by hexane, acetone and methanol extracts (99.26, 98.24 and 97.98 % respectively) (Table16 a).

Acetone extracts of *V. negundo* also recorded lower ECD of 1.344 per cent. Hexane and acetone extracts of *V. negundo* recorded less than ten per cent (7.772 to 0.482 and 8.945 to 1.344 %) at all concentrations. Hexane extracts (2 and 4%), acetone extracts (2%), water extract (6%) and methanol extract (4%) were on par. Control treatments (solvents alone) recorded a significant variation in ECD when *S. litura* larvae were reared on castor. In the control treatment, highest ECD of 76.55 per cent was observed in acetone followed by hexane, water and methanol (64.94, 52.89 and 49.11 %) respectively.

Table 15. Effect of different solvent extracts of *V. negundo* on ECI of *H. vigintioctopunctata*

Concentration (%)	ECI (%)			
	Hexane extract	Acetone extract	Water extract	Methanol extract
1	*8.503(4.808)^{b}	1.432(3.991)^{bc}	4.811(4.421)^{abc}	11.380(5.113)^{b}
2	4.879(4.434)^{bc}	1.769(4.053)^{bc}	5.684(4.523)^{abc}	12.048(5.176)^{b}
4	5.610(4.512)^{bc}	-0.406(3.787)^{cd}	1.244(3.946)^{bc}	2.745(4.168)^{bc}
6	-7.268(2.690)^{e}	-0.857(3.265)^{cde}	1.624(3.946)^{bc}	-5.20(3.085)^{d}
Control	34.830(5.944)^{a}	26.928(5.237)^{a}	22.597(4.806)^{ab}	27.412(5.283)^{a}
% Reduction over control	120.87	103.19	92.83	118.97

*Figures in parathesis denote square root transformation. In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

Table 16. Effect of different solvent extracts of *V. negundo* on Efficiency of Conversion of Digested food to body substances (ECD) of *S. litura* on castor, semi-synthetic diet and banana

Hosts	Concentration %	ECD (%)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
**Castor leaf	1	*7.772(3.925)^{cdef}	7.306(3.839)^{cdef}	10.998(4.314)^{cde}	22.70(5.478)^{c}
	2	5.323(3.599)^{def}	3.565(3.311)^{def}	12.084(4.409)^{cd}	10.376(4.237)^{cde}
	4	2.886(3.189)^{def}	8.945(4.063)^{cdef}	8.187(3.491)^{cdef}	3.930(3.402)^{def}
	6	0.482(2.521)^{g}	1.344(2.413)^{f}	4.841(3.951)^{def}	0.991(2.702)^{g}
	Control	64.94(8.089)^{b}	76.55(8.778)^{a}	52.89(7.307)^{bc}	49.11(7.043)^{bc}
Semi-synthetic diet	1	14.070(3.871)^{bcd}	19.405(4.491)^{bc}	16.675(4.257)^{bc}	11.681(3.617)^{bcde}
	2	14.375(3.910)^{bcd}	15.046(4.061)^{bc}	12.846(3.690)^{bcde}	7.439(2.989)^{defg}
	4	16.164(4.201)^{bc}	10.143(3.403)^{bcd}	9.203(3.240)^{bcdef}	7.878(2.092)^{defg}
	6	6.152(2.521)^{defg}	4.220(2.276)^{fgh}	15.989(3.983)^{bc}	0.111(1.217)^{g}
	Control	40.152(6.376)^{a}	48.473(6.998)^{a}	29.695(5.495)^{b}	32.601(5.753)^{b}
Banana leaf	1	45.65^{b}	47.92^{b}	36.10^{bcde}	17.86^{cde}
	2	26.62^{cd}	51.57^{ab}	23.07^{defgh}	20.22^{cde}
	4	20.09^{cde}	14.07^{defgh}	5.83^{fgh}	-14.13^{ghi}
	6	-6.52^{fghi}	5.76^{fgh}	-17.22^{i}	-19.93^{i}
	Control	36.135^{bcde}	36.095^{bcde}	63.90^{a}	55.920^{ab}

*Figures in parathesis denote square root transformation. In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT. ** Separate CRD analysis was done for castor, semi-synthetic diet and banana

Control treatments (solvents alone) recorded comparatively lower ECD in *S. litura* larvae reared on semi-synthetic diet when compared to that in castor. There was significant difference in ECD between different solvents (control). Higher ECD values were recorded by the solvents, hexane and acetone (40.152 and 48.473%) while, water and methanol recorded significantly lower ECD (29.695 and 32.601 %). Lowest ECD was recorded by the methanol extract at six per cent concentration (0.111 %) followed by the acetone extracts at the same concentration with a lower ECD of (4.22%). Hexane extract (6 %) was on par with methanol extract at two and four per cent concentrations (Table 16). Methanol extract at six per cent resulted in 99.66 per cent reduction in ECD and was followed by acetone extract with 91.29 per cent reduction in ECD (Table 16a).

In case of *S. litura* larvae reared on banana, significant variation was observed in control treatments (solvents alone). Highest ECD of 63.90 was observed in water and lowest of 36.135 and 36.095 in hexane and acetone respectively. However, hexane and acetone extracts were on par. Experiments done on castor, semi-synthetic diet and banana revealed that ECD of *S. litura* varied with the host food material and the solvents (control). All the *V. negundo* extracts at higher concentrations recorded lower ECD when compared to respective controls (solvents alone) indicating its effect in reducing ECD. Growth was completely inhibited and instead of weight gain, loss of weight was observed in methanol extracts at four and six per cent (-14.13 and -19.93 %), water extracts at six per cent (-17.22%) and hexane extracts at six per cent (-6.52%). Lower concentrations of acetone (1 and 2%) and hexane (1%) extracts recorded higher ECD (47.92, 51.57 and 45.65 %) when compared to respective control (solvents alone).

Irrespective of the host food material, methanol extracts at six per cent recorded lowest ECD in *S. litura*. Unlike in castor and semi-synthetic diet, water extracts of *V. negundo* at six per cent concentration recorded lowest ECD in larvae reared on banana.

Table 16 a. Reduction in ECD of *S. litura* as influenced by different solvent extracts over control

Solvent extracts (6% concentration)	Reduction in ECD over control (%)		
	Castor	Semi-synthetic diet	Banana
Hexane	99.26	84.68	118.04
Acetone	98.24	91.29	84.04
Water	90.85	46.16	126.95
Methanol	97.98	99.66	135.64

Table 17. Effect of different solvent extracts of *V. negundo* on ECD of *H. vigintioctopunctata*

Concentration (%)	ECD (%)			
	Hexane extract	Acetone extract	Water extract	Methanol extract
1	8.591(4.841)^{b}	1.452(4.022)^{bc}	4.886(4.456)^{b}	11.560(5.153)^{ab}
2	4.921(4.464)^{b}	1.795(4.084)^{bc}	5.732(4.554)^{b}	12.231(5.216)^{ab}
4	5.673(4.544)^{b}	-0.413(3.816)^{bc}	1.270(3.977)^{bc}	2.799(4.201)^{b}
6	-7.308(2.726)^{c}	-0.866(3.289)^{bcd}	1.635(3.975)^{bc}	-5.280(3.108)^{bc}
Control	35.907(6.034)^{a}	27.491(6.164)^{a}	23.044(4.852)^{a}	27.990(5.338)^{a}
% Reduction over control	120.36	103.16	92.89	118.86

When *S. litura* was reared on banana, methanol, water and hexane extracts of *V. negundo* resulted in more than 100 per cent reduction in ECD (135.64, 126.95 and 118.04 % respectively) (Table 16 a).

4.2.8.2. Effect of different solvent extracts of V. negundo on ECD of H. vigintioctopunctata

There was no significant difference in ECD of *H. vigintioctopunctata* between different solvents in control treatment (Table 17). All the treatments recorded significantly lower ECD when compared to respective control treatments. Lowest ECD (–7.308%) was recorded by hexane extracts at six per cent level and it caused loss of weight in *H. vigintioctopunctata*. Similar results were observed in acetone extracts at four and six per cent (-0.413 and –0.866%) and methanol extracts six per cent (-5.280%) concentrations respectively. All the solvent extracts except methanol extract at one and two per cent levels (11.560 and 12.231 %) recorded less than 10 per cent ECD at lower concentrations. Except water extract, all the solvent extracts at six per cent recorded more than 100 per cent reduction in ECD (Table 17).

4.2.9. Relative Consumption Rate (RCR)

4.2.9.1. Effect of different solvents of V. negundo on RCR of S. litura on castor, semi-synthetic diet and banana

Results on the RCR of *S. litura* on different hosts are presented in Table 18. There was significant variation in RCR values of different treatments. Results of the experiments done to assess the relative consumption rate of different treatments revealed that in all the treatments, RCR values were comparatively higher indicating higher consumption rate. Among the different hosts tested, semi-synthetic diet recorded highest RCR values. Highest RCR of 10.981 was recorded in case of larvae treated with hexane extract at six per cent level on semi-synthetic diet. Lowest RCR values were recorded when the experiment was done on banana. In all the hosts, lowest RCR value of 0.280 was observed by the solvent extracts of acetone at one per cent level in banana. In case of castor,

Table 18. Effect of different solvent extracts of *V. negundo* on Relative Consumption Rate (RCR) of *S. litura* on castor, semi-synthetic diet and banana

Hosts	Concentration %	Relative Consumption Rate			
		*Hexane extract	Acetone extract	Water extract	Methanol extract
**Castor leaf	1	4.472^{abc}	2.950^{cd}	3.224^{bcd}	3.208^{bcd}
	2	3.083^{bcd}	2.856^{cd}	4.944^{abc}	3.795^{abcd}
	4	3.456^{bcd}	3.117^{bcd}	3.817^{abcd}	3.526^{abcd}
	6	4.100^{abc}	3.048^{bcd}	3.050^{bcd}	4.066^{abc}
	Control	7.871^{a}	8.725^{a}	7.240^{a}	7.027^{a}
Semi-synthetic diet	1	7.756^{b}	6.443^{b}	9.056^{b}	7.735^{b}
	2	8.200^{b}	7.570^{b}	14.156^{a}	10.455^{ab}
	4	8.469^{b}	7.367^{b}	8.111^{b}	9.164^{b}
	6	10.981^{ab}	10.642^{ab}	8.419^{b}	6.703^{b}
	Control	6.721^{b}	10.411^{ab}	7.375^{b}	4.261^{bc}
Banana leaf	1	1.457^{a}	0.280^{c}	1.577^{a}	1.303^{ab}
	2	0.692^{bc}	1.189^{ab}	1.128^{ab}	1.580^{a}
	4	0.692^{bc}	0.848^{abc}	1.237^{ab}	1.305^{ab}
	6	0.790^{bc}	0.580^{bc}	1.081^{ab}	0.946^{abc}
	Control	1.110^{ab}	1.503^{a}	0.881^{abc}	0.854^{abc}

In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT. ** Separate CRD analysis was done for castor, semi-synthetic diet and banana

Table 18a. Reduction in RCR of *S. litura* as influenced by different solvent extracts over control

Solvent extracts (6% concentration)	Reduction in RCR over control (%)		
	Castor	Semi-synthetic diet	Banana
Hexane	47.91	-63.38	28.83
Acetone	65.07	-2.21	61.41
Water	57.87	-14.16	-22.70
Methanol	42.13	-57.31	-10.77

highest RCR values were recorded by aqueous extracts at two per cent level (14.156) .

Acetone extract at six per cent concentration recorded maximum reduction in RCR (65.07 and 61.41 %) over control in case of larvae reared on both castor and banana (Table 18 a).

4.2.9.2. Effect of different solvents of V. negundo on RCR of H. vigintioctopunctata

Among the different solvents, water at one per cent concentration recorded highest RCR with a mean value of 8.075. It was followed by the same solvent at two per cent concentration with a mean value of 6.686. The solvent extracts of methanol also recorded higher RCR at one and two per cent levels with mean consumption rates of 6.117 and 6.044 respectively and were on par with each other. These treatments were also on par with water extracts at four per cent level and hexane extracts at two per cent levels with mean RCR values of 6.128 and 5.875.

Lowest RCR value was recorded by acetone 6 per cent concentration with a mean value of 2.607 and was followed by methanol extracts at four per cent level with a mean value of 3.566 (Table 19). Highest reduction in RCR over control was recorded by acetone extract (50.05 %) followed by hexane and methanol extracts (42.17 and 33.89 % respectively). (Table 19).

4.2.10. Larval growth

Growth of larvae in different treatments was examined on fresh weight basis and results presented in Tables 20 to 21.

4.2.10.1. Effect of different solvent extracts of V. negundo on larval growth of S. litura on castor, semi-synthetic diet and banana

Results on the effect of *V. negundo* extracts on the larval growth of *S. litura* are presented in Tables 20 and 20a.

Castor leaves when applied with solvents alone (control), recorded larval growth of 0.035 to 0.039 g day^{-1}. Between the solvents, there was

no significant difference in larval growth. In case of semi-synthetic diet, larval growth was comparatively higher with a range of 0.039 to 0.073 g day^{-1} in control treatments. Highest larval growth of 0.073 g day^{-1} was observed in the control treatment with acetone. When compared to all the three hosts, in the control treatment, banana recorded lowest larval growth with a range of 0.01 to 0.014 g day^{-1} and were on par with each other.

A reducing trend in larval growth was observed at higher concentrations in treatments with castor and semi-synthetic diet (except aqueous extracts). In case of castor, hexane and acetone extracts at all its concentrations caused very low larval growths ranging from 0.01 to 0.001 and 0.016 to –0.003 g day^{-1}. Lowest larval growth of –0.003 g day^{-1} was recorded by the treatment with acetone extracts at six per cent concentration which caused loss of weight in *S. litura*. Hexane and methanol extracts (6%) recorded lower larval growth (0.001 g/day) and were on par with each other. No significant difference in growth was observed between hexane extract (6%), acetone extract (2%) and methanol extract (4%). Highest reduction in growth of *S. litura* over control (108.11 %) was recorded by acetone extract followed by hexane and methanol extracts (97.22 and 97.44 % respectively) at six per cent concentration (Table 20 a).

In case of semi-synthetic diet, complete inhibition of larval growth was observed by the treatment with methanol extracts at six per cent level. Methanol extracts at lower concentrations itself (1, 2 and 4%) recorded lower larval growth (0.033, 0.025 and 0.011g day^{-1}) and were on par with the solvent extracts of water and acetone at four and six per cent levels and hexane at six per cent levels (0.019, 0.032, 0.027, 0.022 and 0.035 g day^{-1}) respectively. Cent per cent reduction in larval growth was recorded by methanol extract at six per cent concentration. Larval growth was reduced to 69.86 per cent when the larvae were treated with acetone extract (6%) (Table 20 a).

When the experiment was done on banana, all treatments except aqueous extracts at two per cent level were significantly different from the control treatments (solvents alone). Significant growth inhibition and weight loss was recorded by methanol extracts at four and six per cent levels (-0.002 and -0.003g

Table 19. Effect of different solvent extracts of *V. negundo* on Relative Consumption Rate (RCR) of *H. vigintioctopunctata*

Concentration	Hexane extract	Acetone extract	Water extract	Methanol extract
1	5.523^{abcd}	2.816^{d}	8.075^{a}	6.117^{abc}
2	5.875^{abc}	5.397^{abcd}	6.686^{ab}	6.044^{abc}
4	5.028^{bcd}	5.517^{abcd}	6.128^{abc}	3.566^{cd}
6	3.279^{cd}	2.607^{d}	5.047^{bcd}	3.894^{bcd}
Control	5.670 ^{abc}	5.220 ^{abcd}	6.020 ^{abc}	5.890 ^{abc}
% Reduction over control	42.17	50.05	16.16	33.89

Table 20 . Effect of different solvent extracts of *V. negundo* on larval growth of *S. litura* on castor, semi-synthetic diet and banana

Hosts	Concentration %	Larval growth (g/day)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
**Castor leaf	1	*0.01^{cdefg}	0.009^{cdefg}	0.019^{b}	0.017^{bcd}
	2	0.008^{defg}	0.004^{fgh}	0.018^{bc}	0.012^{bcdef}
	4	0.003^{fgh}	0.016^{bcde}	0.007^{efg}	0.004^{fgh}
	6	0.001^{gh}	-0.003^{h}	0.009^{cdefg}	0.001^{gh}
	Control	0.036^{a}	0.037^{a}	0.035^{a}	0.039^{a}
Semi-synthetic diet	1	0.049(0.741)^{ab}	0.049(0.740)^{ab}	0.038(0.734)^{ab}	0.033(0.730)^{bc}
	2	0.044(0.738)^{ab}	0.039(0.734)^{ab}	0.041(0.736)^{ab}	0.025(0.724)^{bc}
	4	0.043(0.737)^{ab}	0.027(0.726)^{bc}	0.019(0.720)^{bc}	0.011(0.715)^{bc}
	6	0.035(0.731)^{bc}	0.022(0.722)^{bc}	0.032(0.730)^{bc}	0.00(0.707)^{c}
	Control	0.044(0.738)^{ab}	0.073(0.757)^{a}	0.041(0.735)^{ab}	0.039(0.734)^{ab}
Banana leaf	1	0.001^{c}	0.010^{a}	-0.003^{d}	-0.002^{d}
	2	0.007^{b}	0.002^{bcd}	0.014^{a}	-0.003^{d}
	4	0.005b^{c}	0.004^{bc}	0.007^{b}	0.007^{b}
	6	0.002^{bcd}	0.001^{c}	0.001^{c}	0.007^{b}
	Control	0.011^{a}	0.014^{a}	0.010^{a}	0.010^{a}

* Figures in parathesis denote square root transformation. In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT. ** Separate CRD analysis was done for castor, diet and banana

day^{-1}) and were equally effective as the water extracts at one per cent level (-0.003 g day^{-1}). Acetone and hexane extracts at six per cent level also recorded lower larval growth (0.001g day^{-1} each). Maximum reduction in larval growth over control was recorded by acetone (92.86%) and water extracts (90%) at six per cent concentration (Table 20 a).

4.2.10.2. Larval growth as influenced by different solvent extracts of V. negundo against H. vigintioctopunctata

Results on the larval growth of *H. vigintioctopunctata* as influenced by different solvent extracts of *V. negundo* are presented in Table 21.

When the brinjal leaves were treated with different solvent extracts (acetone, hexane, water and methanol) of *V. negundo* at various concentrations (1, 2, 4 and 6%), the larval growth of *H. vigintioctopunctata* was found to be reduced with increase in concentrations from one to six per cent.

Instead of weight gain, weight loss happened in the treatments with hexane and methanol extracts at six per cent level (-0.016 and –0.010 g day $^{-1}$). At six per cent level acetone extracts also completely inhibited the larval growth of *H. vigintioctopunctata***.** Aqueous extracts at higher concentrations (4 and 6%) recorded lower larval growths (0.003 and 0.004 g day $^{-1}$) and was on par with methanol extracts at four per cent (0.005 g day $^{-1}$) and acetone extracts at one and two per cent (0.003 g day $^{-1}$each) respectively. Larval weight was reduced to more than 100 per cent on treatment with hexane and methanol extracts (161.54 and 133.33 % respectively). Cent per cent reduction in larval growth was also recorded by acetone extract (Table 21).

Table 20 a. Reduction in larval growth of *S. litura* as influenced by different solvent extracts over control

Solvent extracts (6% concentration)	Reduction in larval growth over control (%)		
	Castor	Semi-synthetic diet	Banana
Hexane	97.22	20.45	81.82
Acetone	108.11	69.86	92.86
Water	74.29	21.95	90
Methanol	97.44	100	30

Table 21. Effect of different solvent extracts of *V. negundo* on larval growth of *H. vigintioctopunctata*

Concentration (%)	Production (g/day)			
	Hexane	Acetone	Water	Methanol
1	0.018(0.735)^{ab}	0.003(0.725)^{bcd}	0.012(0.731)^{abc}	0.023(0.739)^{ab}
2	0.012(07.31)^{abc}	0.003(0.725)^{bcd}	0.014(0.733)^{abc}	0.023(0.739)^{ab}
4	0.012(0.732)^{abc}	0.001(0.723)^{cd}	0.003(0.725)^{bcd}	0.005(0.727)^{bcd}
6	-0.016(0.712)^{e}	0.000(0.723)^{cd}	0.004(0.726)^{bcd}	-0.010(0.717)^{de}
Control	0.026(0.725)^{a}	0.033(0.730)^{a}	0.029(0.727)^{a}	0.030(0.728)^{a}
% reduction over control	161.54	100	86.21	133.33

4.2.11. Relative Growth Rate (RGR)

4.2.11.1. Effect of different solvent extracts of V. negundo on RGR of S. litura on castor, semi-synthetic diet and banana

An experiment was conducted to determine the influence of different solvent extracts of *V. negundo* on RGR of *S. litura* on castor, semi-synthetic diet and banana. Results of the study are presented in Tables 22 and 22a.

In the control (solvents alone) treatment, the lowest RGR was observed when the larvae of *S. litura* were reared on banana (0.262 to 0.349 $gday^{-1}$). Highest growth rate of 0.635 to 1.181 g day^{-1} was observed in larvae reared on semi-synthetic diet. When the larvae were reared on castor, RGR ranged from 0.693 to 0.906 g day^{-1}.

In case of banana, there is no significant difference in RGR in the control treatments where the larvae were treated with different solvents alone. But in semi-synthetic diet, highest RGR of 1.181 $gday^{-1}$was observed in the control treatment with acetone, while in methanol alone treatment recorded a significantly lower RGR of 0.635g day^{-1} indicating its effect on larval growth. When the larvae were reared on castor, the control treatments with acetone and methanol recorded higher RGR of 0.906 and 0.897 g day^{-1} and that of hexane recorded comparatively lower RGR of 0.693 $gday^{-1}$ indicating the influence on RGR.

In all the treatments irrespective of the hosts, except water extracts at four per cent in semi-synthetic diet, RGR showed a declining trend with increase in concentrations from one to six per cent. When the larvae were reared on castor, all the solvent extracts of *V. negundo* recorded significantly lower RGR values when compared to the control treatments.

In case of castor reared *S. litura* larvae, solvent extracts of acetone and methanol at six per cent level completely inhibited growth of larvae and resulted in loss of weight instead of gain (-0.079 and -0.003 $gday^{-1}$). Hexane extracts also recorded lower RGR of 0.026 $gday^{-1}$ at six per cent level. Similar results were also observed when the larvae reared on semi-synthetic diet. Lowest RGR of 0.003 was observed in the methanol extracts at six per cent level. The

Table 22. Effect of different solvent extracts of *V. negundo* on Relative Growth Rate (RGR) of *S. litura* on castor, semi-synthetic diet and banana

Hosts	Concentration %	Relative Growth Rate			
		Hexane extract	Acetone extract	Water extract	Methanol extract
**Castor leaf	1	*0.195^{cdefg}	0.244^{bcdef}	0.281^{bcde}	0.351^{bcd}
	2	0.132^{defgh}	0.093^{efgh}	0.402^{abc}	0.265^{bcde}
	4	0.061^{efgh}	0.247^{bcdef}	0.185^{cdefg}	0.108^{efgh}
	6	0.026^{fgh}	-0.079^{gh}	0.161^{defg}	-0.003^{gh}
	Control	0.693^{abc}	0.746^{ab}	0.906^{a}	0.897^{a}
Semi-synthetic diet	1	0.556(1.056)^{bc}	0.814(1.168)^{ab}	0.796(1.164)^{ab}	0.564(1.061)^{bc}
	2	0.639(1.093)^{ab}	0.744(1.143)^{ab}	0.764(1.153)^{ab}	0.525(1.043)^{bc}
	4	0.693(1.121)^{ab}	0.524(1.043)^{bc}	0.512(1.032)^{bc}	0.217(0.880)^{cd}
	6	0.442(0.980)^{bc}	0.489(1.026)^{bc}	0.634(1.087)^{ab}	0.003(0.853)^{d}
	Control	0.891(1.179)^{ab}	1.181(1.297)^{a}	0.862(1.167)^{ab}	0.635(1.065)^{bc}
Banana leaf	1	0.229^{ab}	0.274^{ab}	0.349^{a}	0.164^{abc}
	2	0.185^{abc}	0.071^{bcd}	0.228^{ab}	0.183^{bcd}
	4	0.150^{bcd}	0.128^{cde}	0.025^{e}	-0.057^{e}
	6	0.038^{cde}	0.035^{e}	-0.094^{e}	-0.093^{e}
	Control	0.262^{ab}	0.349^{a}	0.319^{a}	0.274^{ab}

* Figures in parathesis denote square root transformation. In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT. Separate CRD analysis was done for castor, diet and banana

Table 22a. Reduction in RGR of *S. litura* as influenced by different solvent extracts over control

Solvent extracts (6% concentration)	Reduction in larval growth over control (%)		
	Castor	Semi-synthetic diet	Banana
Hexane	96.25	50.39	85.50
Acetone	110.59	58.59	89.97
Water	82.22	26.45	129.47
Methanol	100.33	99.53	133.94

same solvent extract at four per cent level also recorded lower RGR of 0.217 g day^{-1}. Same trend was also observed in case of larvae reared on banana. Methanol extracts at four and six per cent levels resulted in loss of weight of larvae (-0.057 and -0.093 g day^{-1}) and was on par with water extracts at the same levels (0.025 and -0.094 g day^{-1}) and acetone extracts at six per cent level (0.035 $gday^{-1}$). Hexane extracts at six per cent level recorded lower RGR (0.038 $gday^{-1}$) and was immediately followed by acetone extracts at two per cent level (0.071 $gday^{-1}$).

When the larvae were reared on castor, more than 100 per cent reduction in RGR was recorded by acetone and methanol extracts (110.59 and 100.33 %). Methanol extract recorded highest reduction in RGR over control (99.53 and 133.94 %) when the larvae were reared on semi-synthetic diet and banana. Banana reared larvae recorded 129.47 per cent reduction in RGR on treatment with water extract (Table 22 a).

4.2.11.2. Efficacy of different solvent extracts in reducing RGR of H. vigintioctopunctata

Among the different solvent extracts, highest growth rate was observed in methanol extract at one and two per cent levels with mean growth rates of 0.509 and 0.516g day^{-1} respectively. These treatments were on par with each other. This was then followed by hexane extract at one per cent concentration with a mean growth rate of 0.383 $^{-1}$. Solvent extracts of hexane and methanol at six per cent concentration effectively reduced the growth rate with mean values -0.266 and -0.231 g day^{-1} respectively (negative value indicates loss of weight instead of gain). This was then followed by acetone extracts at six per cent concentration with a mean growth rate of -0.050 (negative value was due to loss of weight instead of gain). Solvent acetone at all its concentrations recorded lower growth rates of 0.049, 0.045 and -0.023 at one, two and four per cent levels. These were on par with water extracts at four per cent level with a mean growth rate of -0.006 (negative value denotes loss of weight instead of weight gain). All the solvent extracts (hexane, acetone and methanol) except water extract recorded more than

Table 23. Effect of different solvent extracts of *V. negundo* on RGR of *H. vigintioctopunctata*

Concentration (%)	Relative Growth Rate			
	Hexane extract	Acetone extract	Water extract	Methanol extract
1	0.383(1.173)^{ab}	0.049(1.022)^{bcd}	0.366(1.152)^{abc}	0.509(1.229)^{a}
2	0.251(1.118)^{abc}	0.045(1.020)^{bcd}	0.321(1.149)^{abc}	0.516(1.231)^{a}
4	0.249(1.117)^{abc}	-0.023(0.987)^{bcd}	-0.006(0.979)^{bcd}	0.076(1.036)^{abcd}
6	-0.266(0.855)^{d}	-0.050(0.963)^{cd}	0.094(1.034)^{abcd}	-0.231(0.875)^{d}
control	0.462(.981)^{a}	0.602(1.050)^{a}	0.499(0.999)^{a}	0.496(0.998)^{a}
% Reduction over control	157.58	108.31	81.16	146.57

Table 24. Correlation of RGR of *S. litura* in different treatments with RCR, ECI and ECD on different hosts

Hosts	ECI	ECD	RCR
Castor	0.901**	0.844**	0.125
Semi-synthetic diet	0.896**	0.814**	0.082
Banana	0.958**	0.862**	0.204
Correlation coefficient (R^2)	0.638		

Table 25. Correlation of RGR of *H. vigintioctopunctata* in different treatments with RCR, ECI and ECD

Correlation	ECI	ECD	RCR
RGR	0.837**	0.833**	0.625
Correlation coefficitient (R^2)	0.638		

100 per cent reduction in RGR (157.58, 108.31 and 146.57 % respectively) (Table 23).

4.2.11.3 Correlation of RGR of S. litura in different treatments with RCR, ECI and ECD on different hosts

Correlation studies were conducted in order to study the impact of different nutritional indices on the RGR of *S. litura*. From the Table 24, it is clear that the nutritional indices ECI and ECD showed a highly significant positive correlation with RGR irrespective of the hosts on which *S. litura* was reared. When RCR was correlated with RGR, there was no significant correlation. Correlation values obtained were in the range of 0.082 on semi-synthetic diet to 0.204 on banana reared *S. litura*. While in case of the nutritional indices, ECI and ECD, correlation values were in the range of 0.896 to 0.958 and 0.814 to 0.868 respectively.

4.2.11.4 Correlation of RGR of H. vigintioctopunctata in different treatments with RCR, ECI and ECD on different hosts

The nutritional indices ECI and ECD recorded significant positive correlation with RGR (0.837 and 0.833). RCR also recorded a positive correlation with RGR, but was statistically insignificant (0.625) (Table 25).

4.2.12. Insect growth regulator (IGR) activity

In order to determine IGR activity of different solvent extracts of *V. negundo*, an experiment was laid out as per 3.4.7.

Observations on total pupation, malformed pupae, total adult emergence, malformed adults etc. were taken and results are presented in Tables 26 and 27.

4.2.12.1. Efficacy of different solvent extracts of V. negundo in inducing morphogenic malformations against S. litura on castor leaf, semi-synthetic diet and banana leaf

When compared to the three hosts, at lower concentrations, total pupation was highest in semi-synthetic diet followed by banana and castor.

In case of castor, all the solvent extracts recorded lower pupation rates at higher concentrations. Pupation of *S. litura* showed a reducing trend except in case of water extracts at four per cent, methanol extracts at two and four per cent concentrations. Hexane extracts recorded pupation of 60 to 33.33 per cent, acetone extracts 76.67 to 60 per cent, water extracts 86.67 to 66.67 per cent and the methanol extracts recorded a pupation of 63.33 to 50 per cent at one to six per cent concentrations respectively. Highest pupation of 86.67 per cent was recorded by water extracts at one per cent concentration, while lowest pupation was recorded by hexane extracts at six per cent concentration with 33.33 per cent pupation. Methanol and water extracts at four per cent level also recorded lower pupation (40 and 46.67 %).

When the same experiment was done on larvae reared on semi-synthetic diet, lowest pupation was recorded by water extracts at six per cent concentration with 46.67 per cent pupation and highest pupation (93.33%) was recorded by hexane extracts at one per cent concentration. In case of banana, lowest pupation was recorded by solvent extracts of methanol at six per cent concentration with 40 per cent pupation. Acetone at six per cent also resulted in lower pupation of 43.33 per cent.

When *S. litura* larvae were reared on castor, malformed pupae were observed at one per cent concentration of hexane and acetone extracts and highest pupal malformation was observed at six per cent concentration of methanol extract (26.67%) (Plate 5b). Hexane extract at six per cent and acetone extract at four per cent concentrations also recorded higher pupal malformations with 23.33 per cent malformed pupae each (Plates 5a & 5c). In semi-synthetic diet, highest pupal malformation was recorded by the hexane extracts at six per cent concentration (26.67%) (Plate 7a) and was followed by hexane and methanol extracts at four per cent level with a pupal malformation of 16.67 per cent each (Plate 7b). Acetone and aqueous extracts at six per cent concentration resulted in pupal malformation of 10 per cent each (Plates7d & 7e). A comparison was made with the normal pupa (Plate 7c) and the malformed pupae to know the differences (Plate 7). In case of *S. litura* larvae reared on banana, pupal malformation started

at one per cent concentration of solvent extracts of hexane (6.67%), acetone and methanol (3.33% each). Highest pupal malformation was observed at six per cent concentration of hexane extracts (Plate 9a) and four and six per cent concentrations of methanol extracts with 23.33 per cent malformed pupae. Aqueous extract resulted in 20 per cent malformed pupation (Plate 9b).

Total adult emergence was highest (80%) in *S. litura* larvae treated with water extracts at one per cent level on castor and at the same concentration lowest adult emergence (36.67%) was observed by the methanol extracts. In case of hexane extracts, adult emergence showed a reducing trend with increase in concentrations (1 to 6%) and lowest adult emergence (23.33%) was observed at six per cent. Adult emergence was highest (80 - 90%) in case of *S. litura* larvae reared on semi-synthetic diet and it reduced upto 36.67 to 46.67 per cent at six per cent level. Lowest adult emergence was observed (36.67%) at six per cent concentration of methanol extract. All the solvent extracts at six per cent concentration (hexane – 46.67 %, acetone – 40 %, water – 43.33 % and methanol – 36.67%) recorded less than 50 per cent adult emergence. When the *S. litura* larvae reared on banana, all the solvent extracts except methanol at four and six per cent showed a reducing trend in adult emergence with increase in concentrations. At one per cent strength, only water extracts recorded higher adult emergence of 38.33 per cent and none of the solvent extracts (hexane – 43.33 %, acetone – 50%, and methanol – 46.67 %) recorded more than 50 per cent adult emergence. Lowest adult emergence was recorded by hexane and acetone extracts at six per cent concentration with only 30 per cent emergence of adults and was followed by methanol extracts at the same concentration with 33.33 per cent adult emergence.

In case of *S. litura* larvae reared on castor, hexane extracts at one per cent level it self recorded 6.67 per cent malformed adults. Methanol extracts at the same level also recorded adult malformation of 3.33 per cent. Highest adult malformation (30%) was recorded by the methanol extract at six per cent level (Plate 6b). Water extract at four per cent also recorded higher adult malformation of 23.33 per cent (Plate 6a). When the *S. litura* larvae were reared on semi-

Table 26. Morphogenic malformations as influenced by different solvent extracts of *V. neugndo* against *S. litura* on castor, semi-synthetic diet and banana

Hosts	Solvent extracts	Concentration of extract (%)															
		1				2				4				6			
		*Tp	Mp	Tae	Ma	Tp	Mp	Tae	Ma	Tp	Mp	Tae	Ma	Tp	Mp	Tae	Ma
Castor leaf	Hexane	60	3.33	43.33	6.67	66.67	6.67	30	10	53.33	13.33	30	13.33	33.33	23.33	23.33	6.67
	Acetone	76.67	3.33	46.67	0.0	73.33	6.67	50	13.33	60	23.33	53.33	16.67	60	13.33	36.67	6.67
	Water	86.67	0.0	80	0.0	73.33	3.33	63.33	3.33	46.76	02	40	23.33	66.67	20	53.33	16.67
	Methanol	63.33	0.0	63.67	3.33	66.67	6.67	33.33	0.0	40	20	30	20	50	26.67	33.33	30
Semi-synthetic diet	Hexane	93.33	0.0	90	0.0	86.67	0.0	83.33	0.0	53.33	16.67	36.67	23.33	53.33	26.67	46.67	30
	Acetone	83.33	3.33	80	6.67	86.67	6.67	80	6.67	50	10	46.67	13.33	53.33	10	40	16.67
	Water	86.67	0.0	80	0.0	90	0.0	86.67	0.0	76.67	6.67	50	13.33	46.67	10	43.33	10
	Methanol	93.33	0.0	86.67	3.33	96.67	6.67	66.67	10	53.33	16.67	43.33	23.33	56.67	13.33	36.67	16.67
Banana leaf	Hexane	70	6.67	43.33	10	53.33	13.33	36.67	10	50	16.67	36.67	23.33	50	23.33	30	26.67
	Acetone	76.67	3.33	50	6.67	56.67	10	40	16.67	50	10	40	13.33	43.33	20	30	13.33
	Water	96.67	0.0	83.33	0.0	76.67	3.33	70	6.67	76.67	20	56.67	23.33	70	20	50	26.67
	Methanol	63.33	3.33	46.67	6.67	66.67	10	30	13.33	56.67	23.33	43.33	30	40	23.33	33.33	30

* Tp – Total pupation (%)
Mp – Malformed pupation (%)
Tae – Total adult emergence (%)
Ma – Malformed adults (%)

synthetic diet, hexane extracts at six per cent strength recorded highest malformed adults (30 %) (Plate 8a). The same solvent extract at four per cent and methanol extracts at four per cent also recorded higher adult deformation of 23.33 per cent each (Plates 8a & 8b). Larvae reared on banana showed highest adult malformation of 30 per cent with methanol extract at six per cent concentration. Hexane and water extracts at the same level also resulted in higher malformed adults of 26.67 per cent each. Solvent extracts of hexane, water and methanol resulted in higher adult malformations at higher concentrations (Table 26).

Among the host food materials, pupation was highest in case of larvae reared on semi-synthetic diet. At one per cent concentration of solvent extract, water extract recorded highest pupation in both castor and banana, while in semi-synthetic diet, hexane and methanol extracts recorded highest pupation of 93.33 per cent and at six per cent concentration highest pupation was found in water extracts in both castor and banana and lowest in hexane and methanol extracts in castor and in banana also, methanol extracts recorded lowest pupation. But in semi-synthetic diet, water extracts recorded lowest pupation.

Both in castor and banana, total adult emergence was maximum when the larvae were treated with one per cent water extracts, while in semi-synthetic diet, adult emergence was maximum in larvae treated with hexane extracts. At six per cent concentration, both hexane and methanol extracts recorded lowest adult emergence in castor and banana. In case of larvae reared on semi-synthetic diet also methanol extracts recorded lowest adult emergence. In both castor and banana, highest adult malformation was recorded by methanol extracts at six per cent concentration and in both cases, water extract also recorded higher adult deformation. When the larvae were reared on semi-synthetic diet, hexane extracts recorded highest adult deformations and was followed by methanol extracts.

5a. Hexane extract 6%

5b. Methanol extract 6%

5c. Acetone extract 6%

Plate 5. Pupal malformations of *S. litura* reared on castor treated with *V. negundo* extracts

Aqueous extract (6%)

Methanol extract (6%)

Plate 6. Adult malformations *S. litura* reared on castor treated with *V. negundo* extracts

7a. Hexane extract 6%

7b. Methanol extract 6%

7c. Normal pupa

7d. Acetone extract 6%

7e. Aqueous extract 6%

Plate 7. Pupal malformations of *S. litura* reared on semi-synthetic diet treated with *V. negundo* extracts

Hexane extract (6%)

Methanol extract (6%)

Plate 8. Adult malformations *S. litura* reared on castor treated with *V. negundo* extracts on semi-synthetic diet

Hexane extract (6%)

Aqueous extract (6%)

Plate 9. Pupal malformations of S. litura reared on banana treated with *V. negundo* extracts

4.2.12.2. Efficacy of different solvent extracts of V. negundo in inducing morphogenic malformations in H. vigintioctopunctata

Pupation of *H. vigintioctopunctata* showed a reducing trend with increase in concentrations of solvent extracts of *V. negundo* (Table 27). Hexane extracts resulted in 96.67 to 53.33 per cent pupation while acetone extracts recorded 73.33 to 66.67 per cent and water and methanol extracts resulted in 83.33 to 53.33 and 76.67 to 50 per cent pupation respectively. Lowest pupation (50.00%) was recorded by six per cent concentration of methanol extract. None of the solvent extracts resulted in more than 10 per cent pupal malformation. Among the different solvent extracts, highest pupal malformation of 10 per cent was recorded by hexane extracts at four per cent concentration. Emergence of adults also showed a reducing trend with increase in concentrations of solvent extracts except hexane extracts at four per cent concentration and the lowest adult emergence of 43.33 per cent was recorded by hexane extracts at four per cent and water and methanol extracts at six per cent concentrations. Hexane extracts recorded total adult emergence of 96.67 to 53.33 per cent and acetone extracts resulted in 70 to 56.67 per cent emergence of adults while water and methanol extracts recorded adult emergence of 80 to 43.33 and 73.33 to 43.33 per cent respectively.

At one per cent strength, none of the solvent extracts recorded pupal and adult malformations and at two per cent strength, pupal malformations started in methanol and acetone extracts with 3.33 per cent pupal deformation each. At the same level, acetone extracts recorded 6.67 per cent deformed adults, while water and methanol extracts recorded only 3.33 per cent each of malformed adults. None of the solvent extracts recorded more than 15 per cent adult malformation. Highest adult malformation of 13.33 per cent was recorded by hexane extract at six per cent concentration and all other solvent extracts recorded less than 10 per cent deformed adults.

Table 27. Morphogenic malformations as influenced by different solvent extracts of *V. neugndo* against *H. vigintioctopunctata*

Solvent extracts	Concentration of extract (%)															
	1				2				4				6			
	*Tp	Mp	Tae	Ma	Tp	Mp	Tae	Ma	Tp	Mp	Tae	Ma	Tp	Mp	Tae	Ma
Hexane	96.67	0.0	96.67	0.0	93.33	0.0	90	0.0	53.33	10	43.33	6.67	56.67	6.67	53.33	13.33
Acetone	73.33	0.0	70	0.0	73.33	3.33	70	6.67	70	0.0	63.33	0.0	66.67	0.0	56.67	3.33
Water	83.33	0.0	80	0.0	80	0.0	73.33	3.33	66.67	3.33	60	6.67	53.33	6.67	43.33	6.67
Methanol	76.67	0.0	73.33	0.0	76.67	3.33	66.67	3.33	53.33	0.0	46.67	3.33	50	3.33	43.33	3.33

*Tp – Total pupation (%)
Mp – Malformed pupation (%)
Tae – Total adult emergence (%)
Ma – Malformed adults (%)

4.2.13. Longevity

4.2.13.1. Effect of different solvent extracts of V. negundo on longevity of S. litura adult moths

In order to study the life span of *S. litura* adults, an experiment was laid out as per 3.4.8 (a) and the results are presented in Table 28.

There is no significant difference in the longevity of adult moths in the control treatment (solvents alone). All the solvent extracts significantly reduced longevity. A reducing trend in longevity was observed from lower concentrations to higher concentrations except in cases of treatment extracts of acetone and methanol extracts at four per cent level. Average life span recorded in the control treatments (solvents alone) were 5.33 to 6 days. From one to six per cent concentrations, longevity recorded in different solvent extracts were in the range of 2.67 to 1.00 day for hexane extracts, 3.33 to 0.67 day for acetone extracts, 1.67 to 1.00 day for methanol extracts and 4 to 1 day for water extracts respectively.

Maximum reduction in longevity over control (87.43 %) was recorded by acetone extract (6%) followed by methanol and hexane extracts (83.33 and 82.36 % respectively). Water extract also resulted in 81.24 % reduction in longevity of adults of *S. litura* (Table 28).

Among different solvent extracts, highest longevity was observed in water extracts (4 days) at one per cent concentration. Lowest longevity of 0.67 days were observed in acetone extracts at six per cent and methanol extracts at four per cent levels. Hexane extract at four and six per cent, methanol and water extracts at six per cent levels also recorded longer longevity of one day and all were on par with each other.

4.2.13.2. Effect of different solvent extracts of V. negundo on longevity of H. vigintioctopunctata

Significant variation was observed in the life span of epilachna beetles in different solvent extracts (Table 28). Highest longevity of 16 days was observed in water extracts at two per cent concentration and was on par with the control treatments

Table 28. Longevity in days of *S. litura* and *H. vigintioctopunctata* adults as influenced by different solvent extracts of *V. negundo*

Insects	Treatment (%)	Longevity (days)			
		Hexane extract	Acetone extract	Methanol extract	Water extract
****S. litura**	1	*2.67^{bc}	3.33^{b}	1.67^{bcd}	4^{ab}
	2	1.67^{bcd}	2.67^{bc}	2.33^{bc}	2.67^{bc}
	4	1.0^{c}	1.33^{bcd}	0.67^{d}	2.33^{bc}
	6	1.0^{c}	0.67^{d}	1.0^{c}	1.0^{c}
	Control	5.67^{a}	5.33^{a}	5.33^{a}	6.00^{a}
H. vigintioctopunctata	1	5.33^{c}	7.67^{bc}	4.67^{cde}	12.33^{ab}
	2	5.67^{bcd}	8.33^{b}	3.67^{d}	16^{a}
	4	1.33^{e}	5^{cde}	1.33^{e}	7.67^{bc}
	6	1.67^{e}	4.67^{cde}	1.00^{e}	5.33^{c}
	Control	17^{a}	14^{ab}	16^{a}	16^{a}

* In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

** Separate CRD analysis was done for *S. litura* and *H. vigintioctopunctata*

Table 29. Fecundity of *S. litura* and *H. vigintioctopunctata* as influenced by different solvent extracts of *V. negundo*

Insects	Treatment (%)	Fecundity (Number of eggs)			
		Hexane extract	Acetone extract	Methanol extract	Water extract
S. litura	1	199^{bcd}	226^{b}	171.67^{c}	445.67^{ab}
	2	164.33^{cde}	119.33^{d}	30^{def}	227.33^{b}
	4	0^{f}	20.33^{def}	16.67^{e}	13^{e}
	6	0^{f}	0^{f}	0^{f}	7.33^{ef}
	Control	546.33^{a}	532^{a}	563.33^{a}	616.66^{a}
H. vigintioctopunctata	1	17.33^{d}	36^{bcd}	4.67^{d}	61^{bc}
	2	5.0^{d}	29^{cd}	6.67^{d}	64.33^{b}
	4	7.67^{d}	28^{cd}	1.67^{d}	37.33^{bcd}
	6	0.0^{d}	16.67^{d}	0.0^{d}	19.67^{d}
	Control	196.33	185.55	185.00	200.30

* In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

** Separate CRD analysis was done for *S. litura* and *H. vigintioctopunctata*

of hexane, methanol and water. Water extracts at one per cent level also recorded higher longevity of 12.33 days. Lowest longevity was observed in treatments with methanol and hexane extracts at four and six per cent levels (1.33, 1.00, 1.33 and 1.67 days) and were on par with each other. Hexane and water extracts of *V. negundo* at six per cent resulted in maximum reduction in longevity (90.18 and 93.75 % respectively) of *H. vigintioctopunctata* over control (Table 28).

4.2.14. Fecundity

Fecundity of the test insects as influenced by different solvent extracts were recorded as per 3.4.9 and the results are presented in Table 29.

4.2.14.1. Effect of different solvent extracts of V. negundo on fecundity of S. litura adult moths

In order to study the reproductive inhibition of different solvent extracts of *V. negundo* against *S. litura*, fecundity of adult moths in different treatments were recorded and the results are presented in Table 29.

Number of eggs laid in the control treatments (solvents alone) were in the range of 532 to 616.66 and there was no significant difference among different solvents. In case of different solvent extracts, highest fecundity was observed in the treatment with water extract at one per cent level (445.67 eggs). A reducing trend in egg laying was observed from one to six per cent concentrations. Hexane extracts at four and six per cent levels completely suppressed the adults from egg laying and were on par with acetone and methanol extracts at six per cent levels. Methanol extracts at four per cent also recorded lower egg laying 16.67 eggs and was on par with water extracts at four and six per cent levels (13 and 7.33 eggs). Cent per cent reduction in fecundity of *S. litura* over control was recorded by hexane, acetone and water extracts. Methanol extract also resulted in higher reduction in fecundity (98.81 %) of *S. litura*.

4.2.14.2. Effect of different solvent extracts of V. negundo on fecundity of H. vigintioctopunctata

The number of eggs laid in different treatments were recorded and the results are presented in Table 29**.**

From the Table 29, it is clear that, highest number of eggs were laid in control treatments (solvents alone) and were in the range of 185 to 200.30. In case of solvent extracts of *V. negundo*, at lower concentrations itself, eggs laid were considerably reduced indicating the effect of *V. negundo* extracts in inducing reproductive inhibition of *H. vigintioctopunctata* adults. A reducing trend in egg laying was observed in all the solvent extracts except water, methanol and hexane extracts at two per cent concentration and egg laying was completely inhibited by the solvent extracts of hexane and methanol at six per cent level and were on par with the same extracts at all the levels (1,2 and 4%). Egg laying recorded by the hexane extracts were in the range of 17.33 to 0.00, acetone extracts recorded 36 to 16.67 eggs, methanol extracts 4.67 to 0.00 and water extracts 61 to 19.67 eggs respectively. Number of eggs laid were less than five at all concentrations (1 to 6 %) of methanol extracts. Hexane and methanol extracts of *V. negundo* exhibits strong reproductive inhibition at all the concentrations tested. At six per cent concentration, hexane and water extracts resulted in cent per cent reduction in fecundity of *H. vigintioctopunctata* over control.

4.3. MEDIAN LETHAL DOSE (LD_{50}) OF DIFFERENT SOLVENT EXTRACTS OF *V. NEGUNDO*

LD_{50} of different solvent extracts were calculated as per 3.4.5 and the data were analysed by Probit anlysis using SPSS programme. Results are presented in Tables 30 and 31.

4.3.1. *S. litura*

Lowest LD_{50} of 423 ppm was recorded by methanol extract and was followed by acetone extract with LD_{50} of 517 ppm. Highest LD_{50} was recorded by

Table 30. LD_{50} value of different solvent extracts of *V. negundo* against *S. litura*

Sl. No.	Treatments (solvent extracts)	Heterogenity $\chi 2$	Regression equation	LD_{50} (ppm)	95 % Fiducial limits	
					Lower	Upper
1	Hexane	8.006	Y= -1.441+ 25.632 X	562	466	790
2	Acetone	4.534	Y= - 1.638+ 31.647 X	517	477	570
3	Water	9.698	Y= - 2.088 + 35.361 X	590	504	783
4	Methanol	53.049	Y= - 2. 544 + 60.067 X	423	302	633

Table 31. LD_{50} value of different solvent extracts of *V. negundo* against *H. vigintioctopunctata*

Sl. No.	Treatments (solvent extracts)	Heterogenity $\chi 2$	Regression equation	LD_{50} (ppm)	95 % Fiducial limits	
					Lower	Upper
1	Hexane	6.783	Y= -1.248 + 35.791 X	348	297	406
2	Acetone	1.741	Y= - 0.755 +17.384 X	434	325	651
3	Water	0.738	Y= - 0.806 +12.097 X	666	476	437
4	Methanol	9.714	Y= - 1.171 + 23.094 X	507	361	1100

water extract (590 ppm). Hexane extract also recorded higher LD_{50} of 562 ppm. Among all the four solvent extracts selected, methanol extract was the most effective and toxic one (Table 30).

4.3.2. *H. vigintioctopunctata*

Same experiment was carried out against *H. vigintioctopunctata* in order to determine the most toxic solvent extract in causing mortality to the treated insects. From the Table 31, it is clear that hexane extracts were most toxic with LD_{50} of 348 ppm, while water extracts recorded highest LD_{50} of 666 ppm. Acetone extracts also recorded lower LD_{50} of 434 ppm, while methanol extracts recorded a comparatively higher LD_{50} of 507 ppm (Table 31).

4.4. INSECTICIDAL ACTION OF DIFFERENT SOLVENT EXTRACTS OF *V. NEGUNDO* AGINST *S. LITURA* AND *H. VIGINTIOCTOPUNCTATA*

Insecticidal action of different solvent extracts against third instar larvae of *S. litura* were tested as per 3.5 a. Results of this experiment are presented in Table 32. From the Table 32, it is clear that methanol extracts at six per cent level was the most toxic one when compared to other solvent extracts and it recorded highest mortality of 96.4 per cent. Methanol extracts at lower concentrations (1, 2 and 4%) recorded very low mortalities of less than 50 per cent. Acetone extracts at six per cent level also recorded higher mortality of 60.7 per cent. Hexane extracts recorded lower mortalities at one, two and four per cent levels (17.9, 10.7 and 35.7 %) and at six per cent level recorded 53.6 per cent mean mortality (Table 32). Hexane extract (1%), acetone extract (2%), water extract (2%) and methanol extract (2 and 4%) were on par in their mortality to *S. litura*. Methanol extract at one per cent caused no mortality of *S. litura*. It was on par with one per cent water extract. Similarly four per cent extracts with hexane, acetone and water were also on par.

When the solvent extracts of *V. negundo* were applied against *H. vigintioctopunctata*, highest mortality of 80 per cent was observed in the treatment with hexane extract at six per cent level. It was followed by acetone extracts at the same level with a mean per cent mortality of 60 (Table 32).

Table 32. Insecticidal action of different solvent extracts of *V. negundo* against *S. litura* and *H. vigintioctopunctata*

Insects	Treatment (%)	*Mortality (%)			
		Hexane extract	Acetone extract	Water extract	Methanol extract
**S. litura*	1	17.9 def	10.7 ef	3.5 f	0 f
	2	10.7 ef	17.9 def	13.8 def	21.4 def
	4	35.7 cde	28.6 cde	27.6 cde	21.4 def
	6	53.6 bcd	60.7 abc	51.7 bcd	96.4 a
H. vigintioctopunctata	1	23.33 e	23.33 e	23.33 e	30 cde
	2	36.67 cde	30 cde	30 cde	23.33 e
	4	56.67 bcd	50 bcd	30 cde	36.67 cde
	6	80 a	60 abc	50 bcd	56.67 bcd

* Mean of ten replications

** Separate analysis was done for *S. litura* and *H. vigintioctopunctata* . In a column, means followed by a common letter are not significantly different at 5 per cent level by DMRT

Table 33. Inhibition on the growth of *Metarhizium anisopliae* and *Beauveria bassiana* as influenced by *V. negundo* extracts

Treatments	Dose (%)	Percentage inhibition over control	
		M. ANISOPLIAE	B. BASSIANA
Methanol	6	54.90 a	45.93 bc
Acetone	6	16.20 bcd	15.85 e
Hexane	6	3.04 cd	31.71 cd
Water	6	19.58 bc	12.19 ef
Azadirachtin	0.03	71.06 a	63.01 a
Cypermethrin	0.002	25.97 b	26.83 de
Quinalphos	0.05	68.43 a	55.28 ab

Methanol extract at one per cent level recorded a mean mortality of 30 and it was increased upto 56.67 per cent at six per cent level. Aqueous extracts recorded lower mortality of 23.33 per cent at one per cent level and was on par with methanol extract at two per cent, acetone and hexane extracts at one per cent levels with the same mean per cent mortality each. In case of acetone extracts, highest mortality was observed at six per cent level with 60 per cent mortality.

4.5. EVALUATION OF COMPARATIVE EFFICIENCY OF MICROBIALS AND BOTANICALS ON *S. LITURA* AND *H. VIGINTIOCTOPUNCTATA*

4.5.1. Compatibility of entomopathogens with botanicals and insecticides

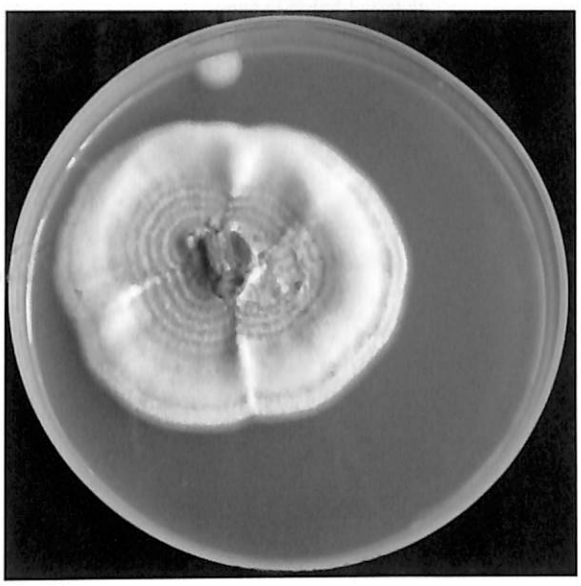
4.5.1.1. Inhibitory effect of V. negundo on the growth of Metarhizium anisopliae and Beauveria bassiana

In order to study the compatibility of *V. negundo* extracts and insecticides on the growth of *M. anispliae* and *B. bassiana* an experiment was carried out as per 3.7 and the results are presented in Table 33.

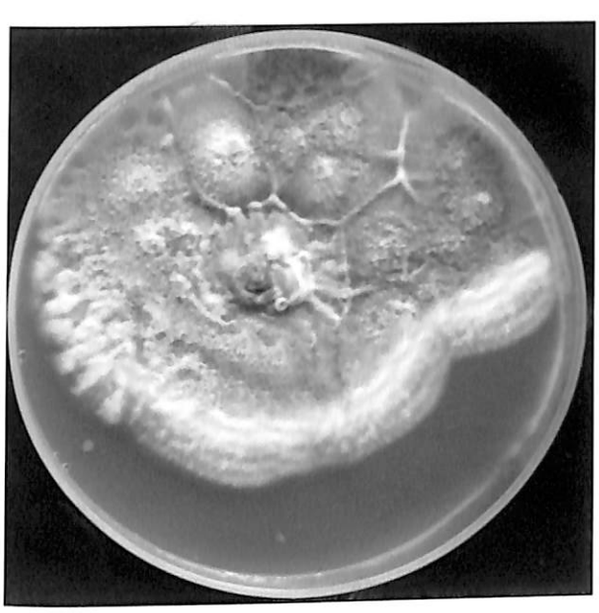
Among different solvent extracts of *V. negundo*, methanol extract at six per cent level resulted in maximum growth inhibition (54.90%) when compared to control (Plates10b&10a). *V. negundo* extract in methanol was on par with azadirachtin and quinalphos (Plates 10f & 10g). Lowest inhibition of 3.04 per cent was obtained by the hexane extract at six per cent level (Plate 10e). Acetone and water extracts at the same level also recorded lower inhibition of 16.20 and 19.58 per cent respectively (Table 33) and (Plates 10d & 10c). Among the insectides, cypermethrin resulted in lowest inhibition of 25.97 per cent (Plate 10h).

Methanol, acetone, hexane and water extracts caused inhibition of 45.93, 15.85, 31.71, and 12.19 % respectively on the growth of *B. bassiana* (Plates 11b, 11d, 11h, & 11c). Azadirachtin caused significantly higher inhibition (63.01 %) (Plate 11f) and lowest by water extract of *V. neugndo* (12.19 %). The insecticide quinalphos resulted in higher inhibition of 55.28 per cent, when compared to control, while the synthetic pyrethroid, cypermethrin resulted in lower inhibition of 26.83 per cent (Plates 11g, 11a & 11e). *M. anisopliae* and *B. bassiana* were

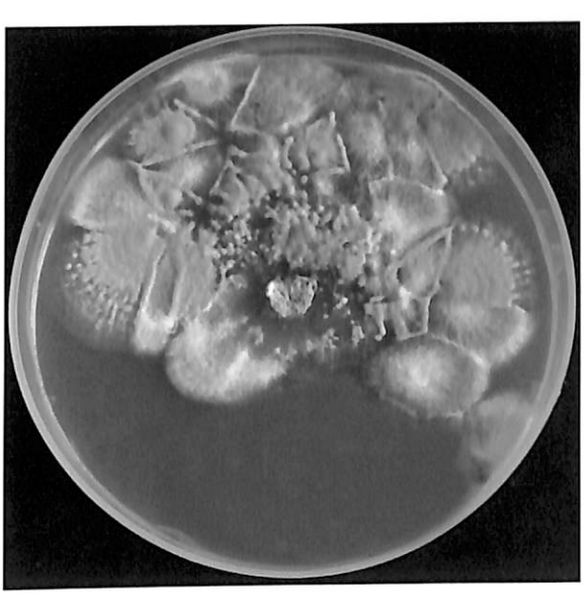
10a. Control – *M. anisopliae*



10b. Methanol extract + *M. anisopliae*



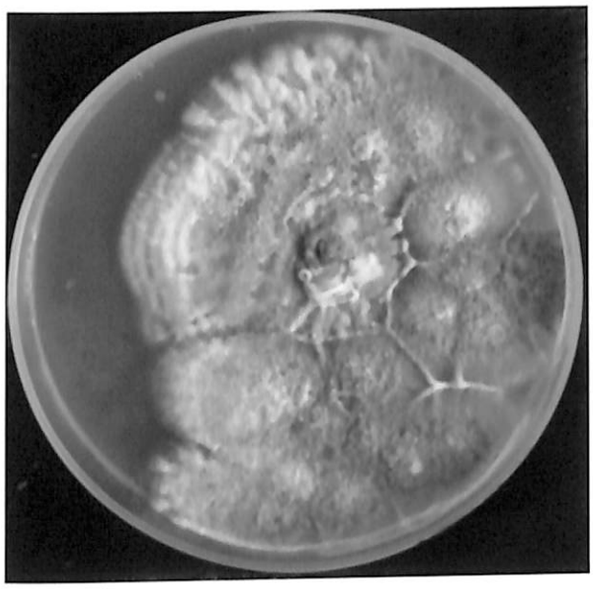
10d. Acetone extract + *M . anisopliae*



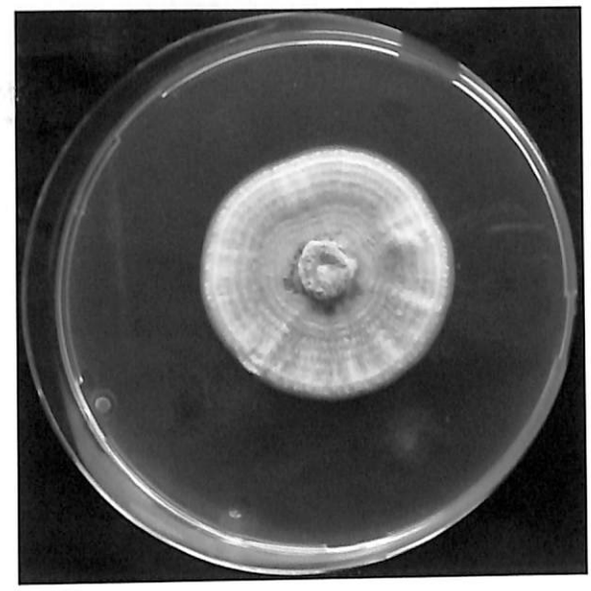
10c. Aqueous extract + *M. anisopliae*

Plate 10. Compatibility of *V. negundo* extracts and insecticides with *Metarhizium anisopliae*

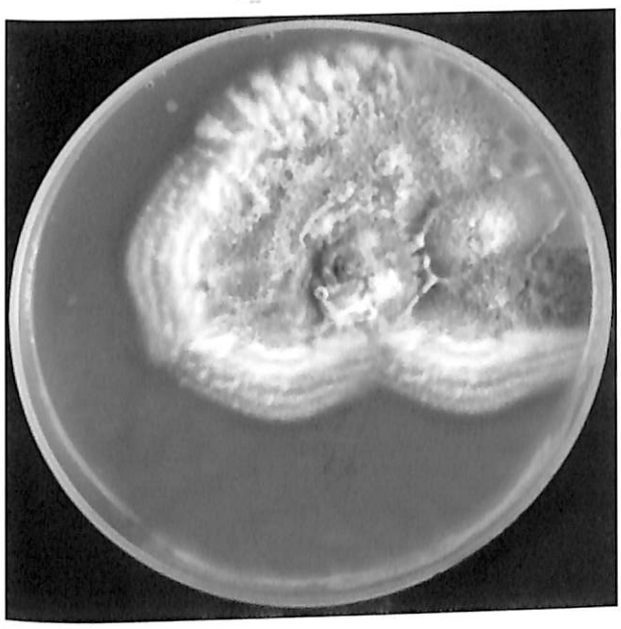
Plate 10 continued…



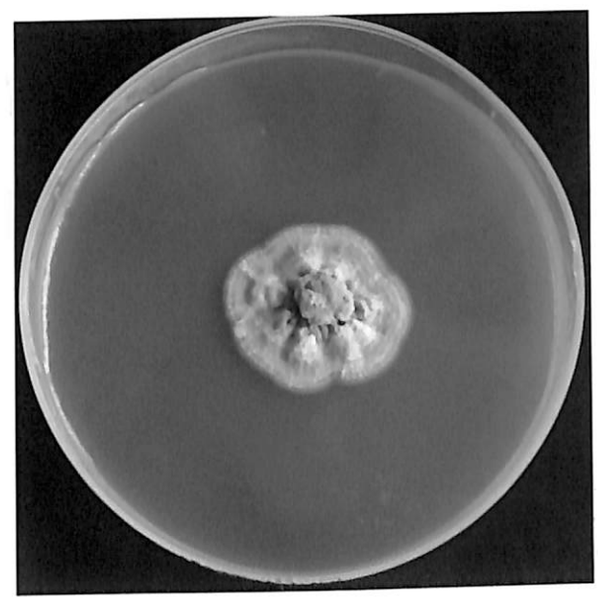
10e. Hexane extract + *M. anisopliae*



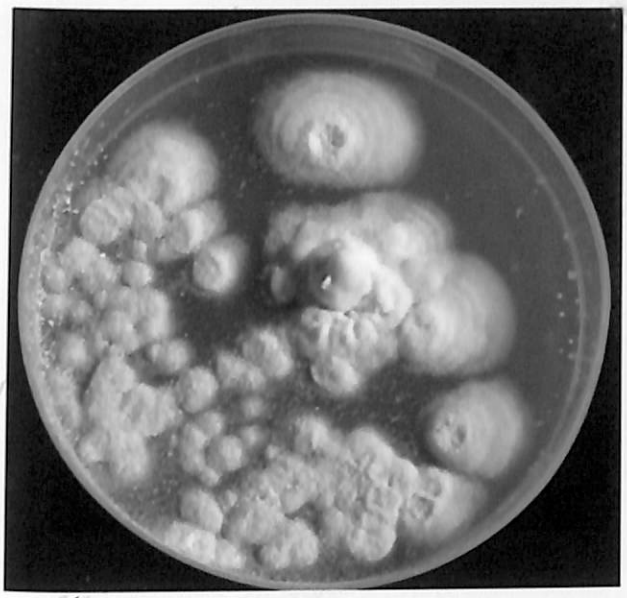
10f. Quinalphos + *M .anisopliae*



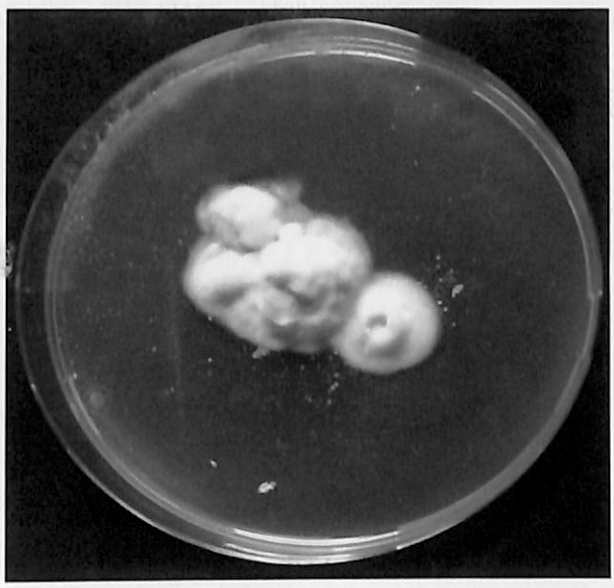
10h. Cypermethrin + *M. anisopliae*



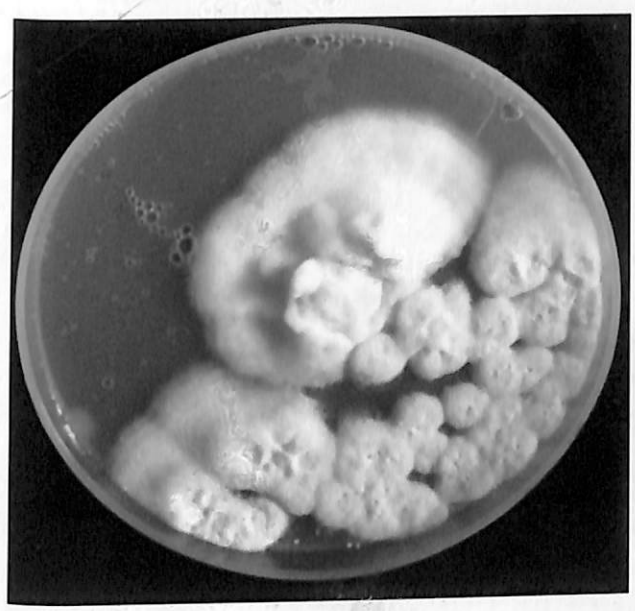
10g. Azadirachtin + *M. anisopliae*



11a. Control – *B. bassiana*



11b. Methanol extract + *B. bassiana*

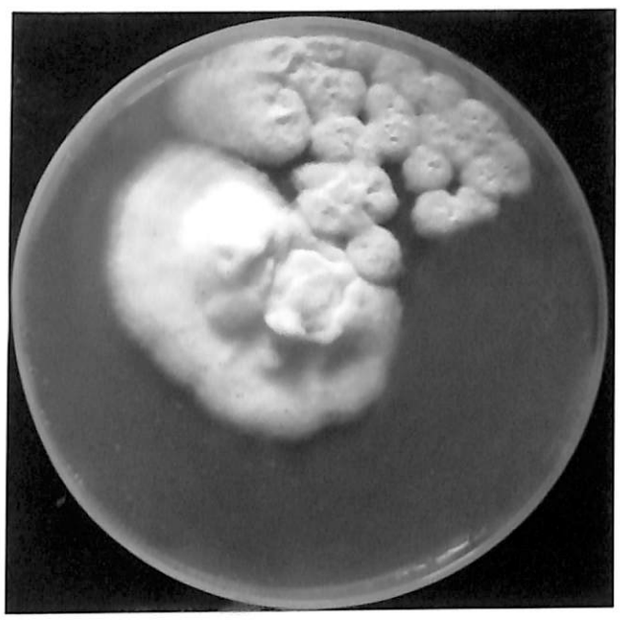


11d. Acetone extract + *B. bassiana*

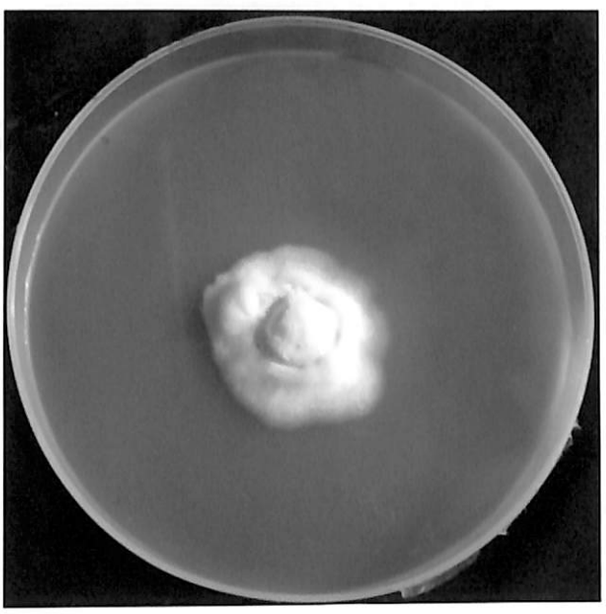
11c. Aqueous extract + *B. bassiana*

Plate 11. Compatibility of *V. negundo* extracts and insecticides with *Beauveria bassiana*

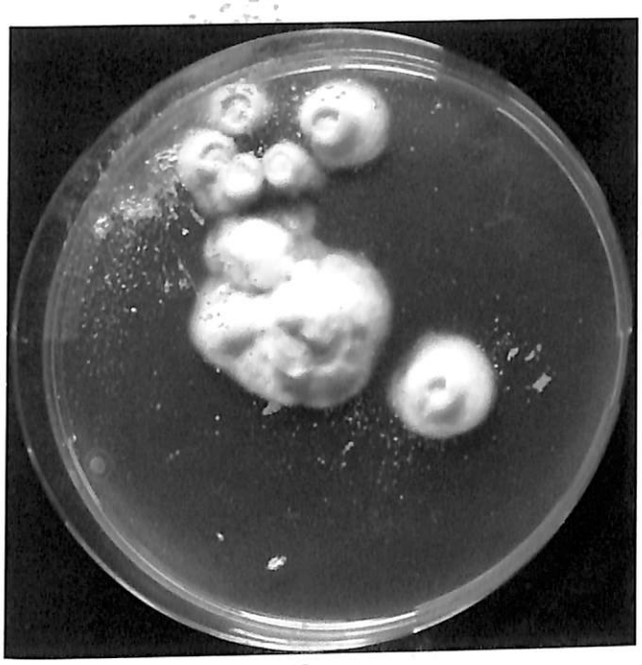
Plate 11 continued…



11e. Cypermethrin + *B. bassiana*



11f. Azadirachtin + *B. bassiana*



11h. Hexane extract + *B. bassiana*

11g. Quinalphos + *B. bassiana*

highly inhibited by azadirachtin (71.06 and 63.01 %).Methanol extract of *V. negundo* caused 54.90 and 45.93 per cent inhibition of *M. anisopliae* and *B. bassiana* respectively. Hexane extract inhibited the growth of *M. anisopliae* by 3.04 per cent while a higher inhibition towards *B. bassiana* (31.71%). Aqueous extract caused 19.58 per cent inhibition for *M. anisopliae* and 12.19 per cent to *B. bassiana*.

4.5.1.2. Compatibility of B. thuringiensis with insecticides and botanicals

There was no inhibition zone developed around the filter paper impregnated with the insecticides and botanicals. Hence it is concluded that all the treatments were highly compatible with the bacteria, *B. thuringiensis* (Plates 12a to 12 h).

4.5.2. Studies on interaction of entomopathogens with *V. negundo* extracts

V. negundo extracts in combination with microbials were evaluated under laboratory conditions. This was done to assess the synergestic/ antagonistic response of combined application of *V. negundo* extract and microbials against *S. litura* and *H. vignitioctopunctata*.

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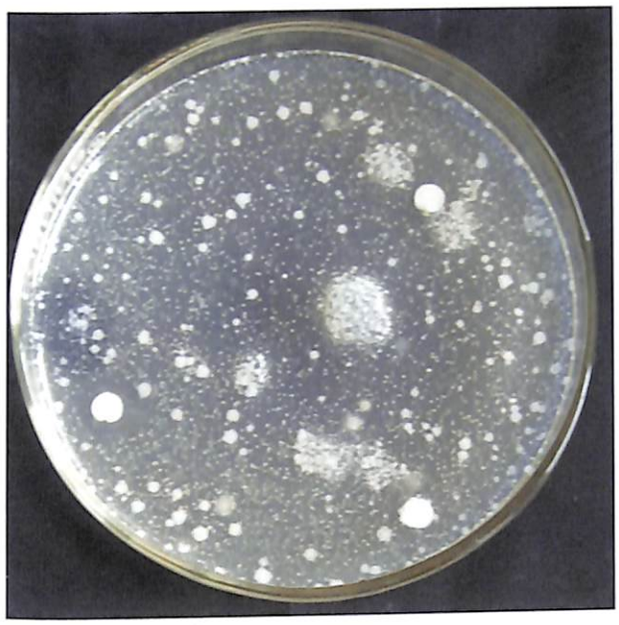
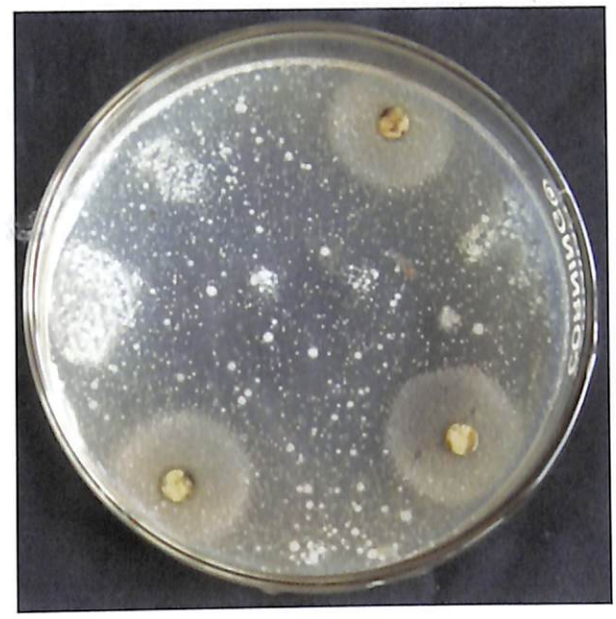
4.5.2.1. M. anisopliae and different solvent extracts of V.negundo against S.litura

An experiment was conducted as per 3.6.3.1 to evaluate the combined efficacy of *M. anisopliae* and different solvent extracts of *V. negundo* against third instar larvae of *S. litura*. Results of combined application are presented in Table 34 and their interaction effects in Table 35.

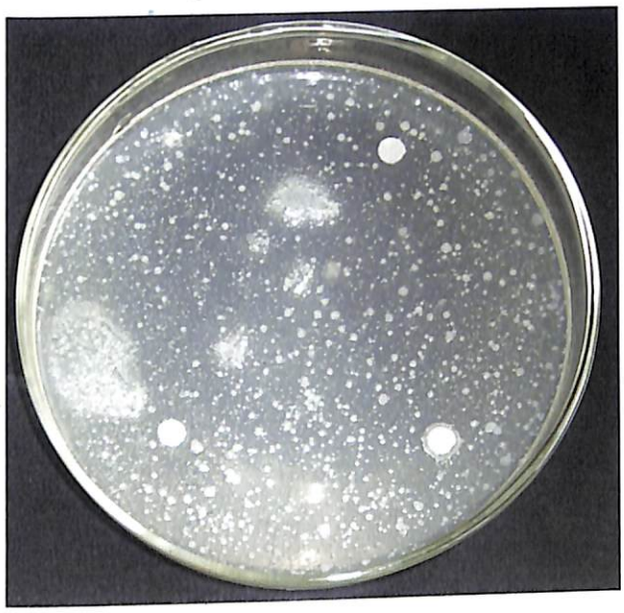
Mortality of *S. litura* was found to increase with increase in exposure period of extracts of *V. negundo*, *M. anisopliae* and their combinations. Highest mortality was observed at 10 days by all treatments and methanol extract of *V. negundo* caused the highest mortality (73.3 %) to *S. litura*. But the combination of methanol extract and *M. anisopliae* reduced the mortality to 40 per cent. Hexane and acetone extracts of *V. negundo* in combination with *M. anisopliae* increased the mortality of *S. litura* from 43.3 to 60 per cent and 40 to 60 per cent



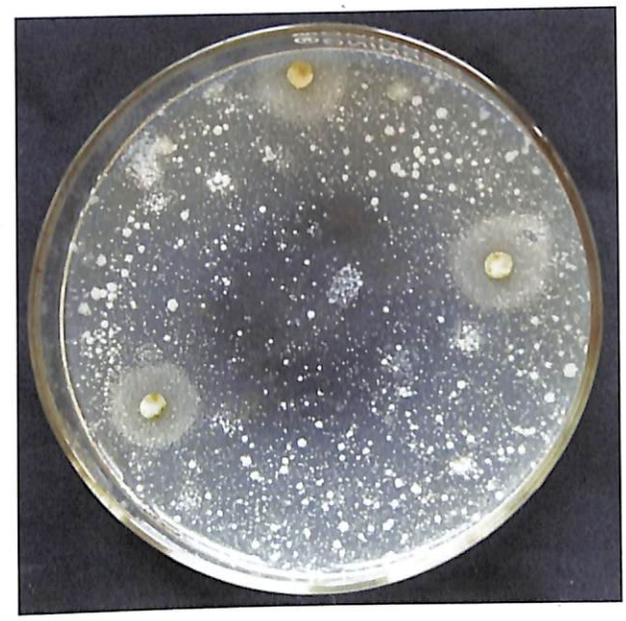
12a. Methanol extract + *B. thuringiensis*



12b. Acetone extract + *B. thuringiensis*



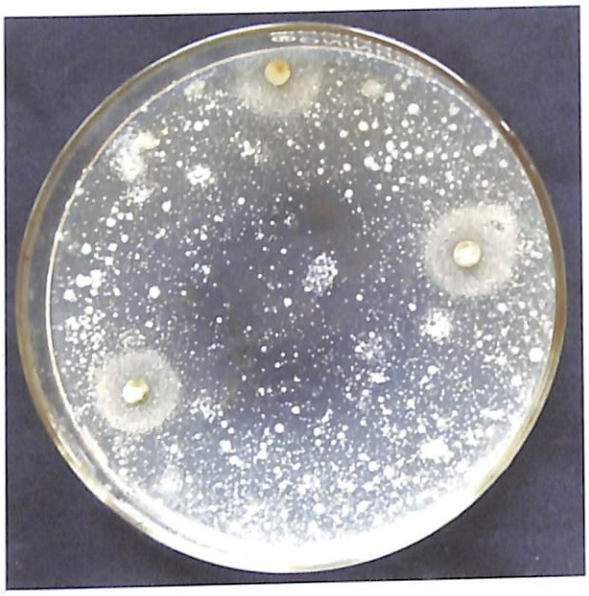
12d. Quinalphos + *B. thuringiensis*



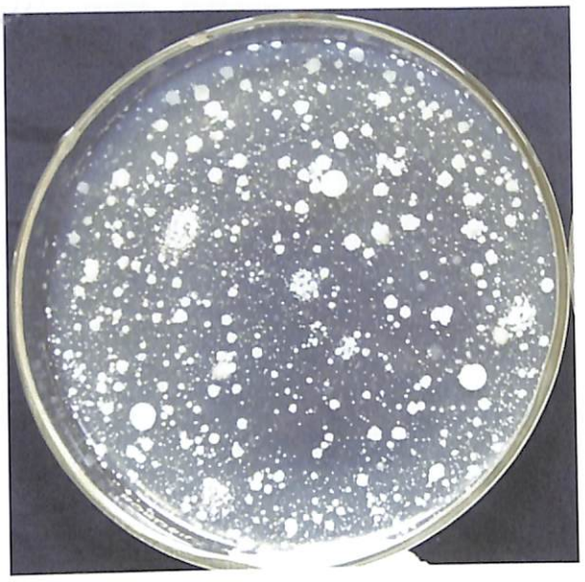
12c. Cypermethrin + *B. thuringiensis*

Plate 12. Compatibility of *V. negundo* extracts and insecticides with *Bacillus thuringiensis*

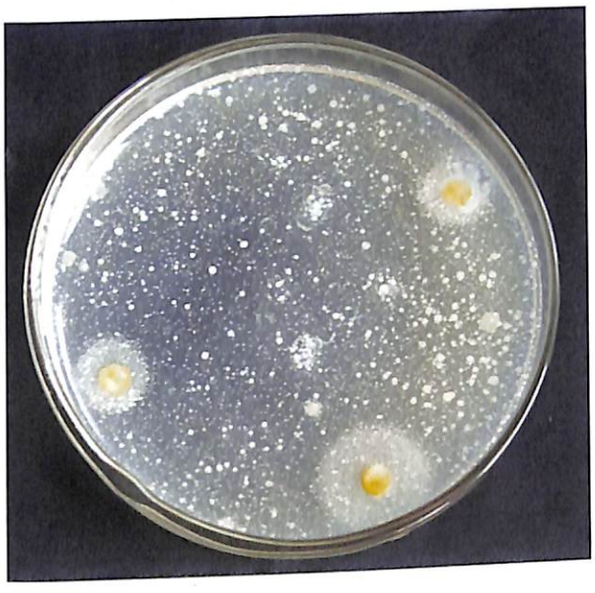
Plate 12 continued…



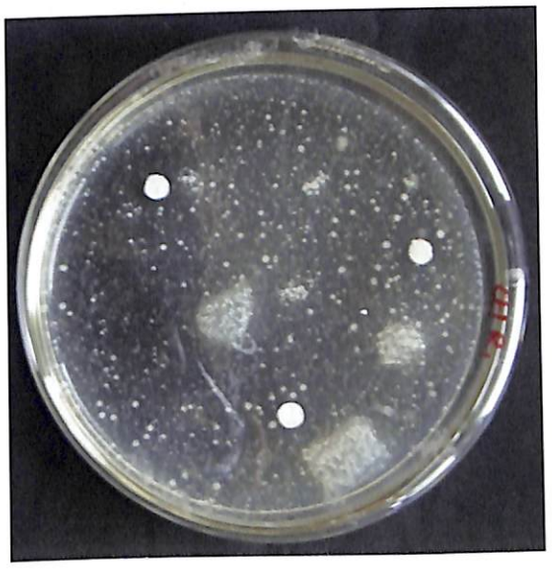
12e. Hexane extract + *B. thuringiensis*



12f. Water extract + *B. thuringiensis*



12h. Azadirachtin + *B. thuringiensis*



12g. Control – *B. thuringiensis*

Table 34. Mortality of third instar larvae of *S. litura* treated with *V. negundo* extracts in combination with *M. anisopliae*

Treatments	Dose (%)	Days after treatment										LT_{50}
		1	2	3	4	5	6	7	8	9	10	
Methanol extract	5	*20	20	43.33	43.33	63.33	73.33	73.33	73.33	73.33	73.33	4.65
Hexane extract	5	0	0	0	0	0	0	20	40	40	40	12.09
Acetone extract	5	0	0	10	10	10	20	30	30	43.33	43.33	11.29
Water extract	5	0	0	0	0	0	0	20	20	53.33	63.33	9.15
Metarhizium anisopliae	$1*10^8$ spores/ml	0	0	0	10	20	20	30	43.33	43.33	63.33	9.37
M.anisopliae+methanol extract	$0.5*10^8$spores/ml+ 2.5	0	0	0	0	0	10	20	40	40	40	11.85
M.anisopliae+hexane extract	$0.5*10^8$spores/ml+ 2.5	20	20	30	40	40	43.33	50	60	60	60	7.10
M.anisopliae+acetone extract	$0.5*10^8$spores/ml+ 2.5	0	0	23.33	23.33	40	40	56.67	56.67	60	60	7.38
M.anisopliae+water extract	$0.5*10^8$spores/ml+ 2.5	0	0	0	0	6.67	10	40	56.67	56.67	63.33	8.70

* per cent mortality

Table 35. Effect of combination of *M. anisopliae* and different solvent extracts of *V. negundo* against *S. litura*

Botanicals	Mortality (%)			Effect	Synergestic coefficient and effect
	M_b	M_f	$M_b + M_f$		
Methanol extract	73.33 (17.14)	63.33 (45.76)	40	No synergism	
Hexane extract	40 (29.30)		60	Sub additive synergism	-0.017 (SMS)
Acetone extract	43.33 (25)		60	Sub additive synergism	-0.017 (SMS)
Water extract	63.33 (17.39)		63.33	Supplimental synergism	-0.016 (SMS)

Figures in paranthesis are theoretical mortality at half dose of fungus and botanicals.

SMS- Sub Multiplicative Synergism

M_b – Mortality (%) caused by *V. negundo* extracts

M_f - Mortality (%) caused by fungus (*Metarhizium anisopliae*)

respectively. Water extract showed no change in mortality when added with *M. anisopliae*.

Time mortality response studies revealed that the treatment with *M. anisopliae* alone recorded 9.37 days for 50 per cent mortality. Lowest LT_{50} of 4.65 days was observed in methanol extract alone. Combination treatments of *M. anisopliae* with hexane, acetone and water extracts showed comparatively lower LT_{50} values of 7.10, 7.38 and 8.70 days respectively. Treatment with hexane extract alone recorded LT_{50} of 12.09 days whereas in combination treatment of the same with *M. anisopliae*, the LT_{50} was reduced to 7.10 days. Same trend was observed in case of acetone extract also. Treatment with acetone extract recorded an LT_{50} of 11.29 days, while in case of combination treatment with *M. anisopliae*, number of days needed for killing 50 per cent population of the test insects was reduced to 7.38 days. Water extracts alone recorded higher LT_{50} of 9.15 days where as in combination treatment with *M. anisopliae*, LT_{50} was reduced to 8.70 days. The interaction effects of different treatments are presented in Table 34. Synergism was not at all observed in the treatment combination of *M. anisopliae* with methanol extract. Treatment combinations of hexane and acetone extracts resulted in sub additive synergism. Water extracts with *M. anisopliae* recorded supplementary synergism. The synergestic coefficients observed were –0.017, -0.017 and –0.016 respectively showing sub multiplicative synergism (Table 35).

4.5.2.2. Beauveria bassiana and different solvent extracts of V. negundo against S. litura

Results of the experiment on the combined efficacy of *B. bassiana* and *V. negundo* solvent extracts are presented in Tables 36 and 37.

B. bassiana caused mortality to *S. litura* from eighth day after treatment (56.67%), while in case of combination treatments of *B. bassiana* with methanol extract, mortality started from second day (16.67%) onwards and in case of hexane and acetone extracts with *B. bassiana*, mortality was observed from third day onwards (10%).

Highest mortality was observed in treatments with *B. bassiana* alone and methanol extract alone with a mean per cent mortalities of 73.33 each. Lowest per cent mortality was observed in treatment with hexane extract with a mean per cent mortality of 40. But in combination with *B. bassiana*, the same solvent extract recorded a higher mortality rate of 66.67 per cent. Acetone extract also recorded lower mortality of 43.33 per cent which when combined with *B. bassiana*, mortality was increased upto 53.33 per cent. Both water extracts and the combination of the same with *B. bassiana* resulted in 63.33 per cent mortality and was on par with the combination treatment of methanol extract with *B. bassiana*.

Lowest LT_{50} (4.65) was observed in the treatment with methanol extract alone. Number of days needed for 50 per cent mortality was higher in case of *B. bassiana* alone treatment with an LT_{50} of 9.05 days. But in combination studies, there was a reduction in LT_{50} values. Combination of the fungus with methanol extract resulted in lower LT_{50} of 6.59 days. Treatment combinations of the same fungus with hexane, water and acetone extracts also resulted in lower LT_{50} values of 7.93, 7.92 and 8.63 days respectively. When the insects were treated with hexane extracts alone, the LT_{50} obtained was 12.09 days whereas in the combination treatment with fungus *B. bassiana*, LT_{50} was reduced to 7.93 days. Same trend was observed in case of acetone and water extracts with highest LT_{50} values of 11.29 and 9.15 days when used alone and there was a reduction in LT_{50} upto 8.63 and 7.92 days in case of combination treatments of the same extracts with *B. bassiana*. Water extracts produced an LT_{50} of 9.15 days and it was reduced upto 7.94 days in combination treatment with *B. bassiana*. All these combinations in which the LT_{50} values were lowered are catagorised under temporal synergism (Table 36).

Interaction effect of combining *B. bassiana* with sub lethal doses of different plant extracts of *V. negundo* is presented in Table 36. Treatment combinations of methanol and water extracts with *B. bassiana* resulted in supplemental synergism. These treatments recorded synergestic coefficients of –0.015 and –0.016 exhibiting sub multiplicative synergism. Sub additive synergism

Table 36. Mortality of third instar larvae of *S. litura* treated with *V. negundo* extracts in combination with *B. bassiana*

Treatments	Dose (%)	Days after treatment										LT_{50}
		1	2	3	4	5	6	7	8	9	10	
Methanol extract	5	*20	20	43.33	43.33	63.33	73.33	73.33	73.33	73.33	73.33	4.65
Hexane extract	5	0	0	0	0	0	0	20	40	40	40	12.09
Acetone extract	5	0	0	10	10	10	20	30	30	43.33	43.33	11.29
Water extract	5	0	0	0	0	0	0	20	20	53.33	63.33	9.15
Beaveria bassiana	$1*10^8$ spores/ml	0	0	0	0	0	0	0	56.67	66.67	73.33	9.049
B. bassiana +methanol extract	$0.5*10^8$spores/ ml+ 2.5	0	16.67	16.67	43.33	43.33	53.33	63.33	63.33	63.33	63.33	6.19
B.bassiana+hexane extract	$0.5*10^8$spores/ ml+ 2.5	0	0	10	20	30	30	40	56.67	56.67	66.67	7.30
B.bassiana+acetone extract	$0.5*10^8$spores/ ml+ 2.5	0	0	10	20	20	40	40	40	53.33	53.33	8.63
B. bassiana+water extract	$0.5*10^8$spores/ ml+ 2.5	0	0	0	20	20	40	53.33	53.33	53.33	63.33	7.92

* per cent mortality

Table 37. Effect of combination of *B. bassiana* and different solvent extracts of *V. negundo* against *S. litura*

Botanicals	Mortality (%)			Effect	Synergestic coefficient and effect
	M_b	M_f	$M_b + M_f$		
Methanol	73.33 (17.14)	73.33 (55.20)	63.33	Supplimental synergism	-0.015
Hexane	40 (29.30)		66.67	Sub additive synergism	-0.016 (SMS)
Acetone	43.33 (25)		53.33	Sub additive synergism	-0.02 (SMS)
Water	63.33 (17.39)		63.33	Supplimental synergism	-0.016 (SMS)

Figures in paranthesis are theoretical mortality at half dose of fungus and botanicals.

SMS- Sub Multiplicative Synergism

M_b – Mortality (%) caused by *V. negundo* extracts

M_f - Mortality (%) caused by fungus (*Beauveria bassiana*)

was observed in combination treatments of hexane and acetone extracts with *B. bassiana* (Table 37).

4.5.2.3. Combination of Bacillus thuringiensis and solvent extracts of V. negundo against S. litura

A perusal of data on the combined action of Delfin and sub lethal doses of *V. negundo* extracts against third instar larvae of *S. litura* are presented in Tables 38 and 39.

Highest mortality was observed in the combination treatment with Delfin and hexane extracts resulting in 89.66 per cent mortality. In case of treatment with Delfin alone caused a mortality of only 62.06 per cent (Plate 13a). All the combination treatments of Delfin with hexane, methanol, acetone and water extracts recorded higher mortalities, when compared with the treatment in which Delfin was applied alone. In case of treatment combinations of methanol, acetone and water extracts with Delfin, mortalities obtained were 75.87, 65.52 and 65.52 per cent respectively. Plant extracts alone gave 37.93 to 72.41 per cent mortality only. Lowest mortality was observed in treatment with hexane extract alone with 37.93 per cent mortality and it was increased upto 89.66 per cent when combined with Delfin. Same trend was observed in case of acetone extract in which the same solvent extracts alone caused only 41.38 per cent mortality and it was increased upto 65.52 per cent when applied along with Delfin. Similarly methanol and water extracts in combination with Delfin recorded higher mortalities of 75.87 and 65.52 per cent when compared to those applied alone. Time mortality response studies revealed that lowest LT_{50} of 4.64 days was observed in the combination treatment of methanol extract and Delfin and was on par with methanol extract alone with the same LT_{50} value. Treatment with Delfin alone had taken 7.79 days for causing 50 per cent mortality of *S. litura*, but the number of days was reduced considerably in all the combination treatments with 4.60 (methanol+Delfin), 5.58 days (Delfin+hexane), 7.22 (Delfin+water) and 7.80 (Delfin+acetone) days respectively.

Table 38. Mortality of third instar larvae of *S. litura* treated with *V. negundo* extracts in combination with *Bacillus thuringiensis*

Treatments	Dose (%)	Days after treatment										LT_{50}
		1	2	3	4	5	6	7	8	9	10	
Methanol extract	5	*20	20	43.33	43.33	63.33	73.33	73.33	73.33	73.33	**72.41	4.65
Hexane extract	5	0	0	0	0	0	0	20	40	40	37.93	12.09
Acetone extract	5	0	0	10	10	10	20	30	30	43.33	41.38	11.29
Water extract	5	0	0	0	0	0	0	20	20	53.33	62.06	10.63
Delfin	0.2	0	10	10	20	20	43.33	43.33	53.33	63.33	62.06	7.79
Delfin + methanol extract	0.1 + 2.5	23.33	43.33	43.33	43.33	50	53.66	60	76.67	76.67	75.87	4.64
Delfin + hexane extract	0.1 + 2.5	13.33	13.33	13.33	13.33	33.33	56.67	76.67	90	90	89.66	5.58
Delfin + acetone extract	0.1 + 2.5	0	0	10	20	30	30	46	46	66.67	65.52	7.80
Delfin+ water extract	0.1 + 2.5	0	0	10	10	20	46.67	66.67	66.67	66.67	65.52	7.22

* per cent mortality

**corrected mortality using Abbot's formula

Table 39. Effect of combination of *B. thuringiensis* (Delfin) and different solvent extracts of *V. negundo* against *S. litura*

Botanicals	*Corrected percentage mortality			Effect	Synergestic coefficient and effect
	M_b	M_{ba}	$M_b + M_{ba}$		
Methanol	72.41 (17.14)	62.06 (45)	75.87	Supplimental synergism	-0.013 (SMS)
Hexane	37.93 (29.30)		89.66	Supplimental synergism	-0.005 (SMS)
Acetone	41.38 (25)		65.52	Sub additive synergism	-0.15 (SMS)
Water	62.06 (17.39)		65.52	Supplimental synergism	-0.015 (SMS)

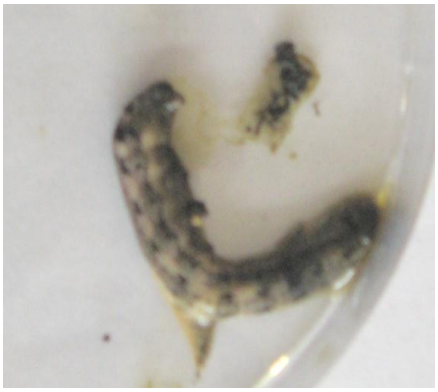
* Corrected mortality using Abbot's formula

Figures in paranthesis are theoretical mortality at half dose of fungus and botanicals.

SMS- Sub Multiplicative Synergism

M_b – Mortality (%) caused by *V. negundo* extracts

M_{ba} - Mortality (%) caused by bacteria (*Bacillus thuringiensis*)



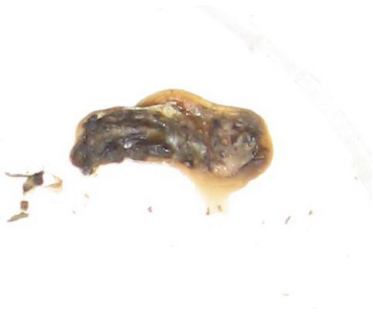
Delfin (0.2 %)

NPV alone

Aqueous extract + NPV

Acetone extract + NPV

Methanol extract + NPV



Hexane extract + NPV

Plate 13. Joint action of *V. negundo* extracts with entomopathogens

Different solvent extracts alone (hexane, acetone and water) resulted in higher LT_{50} of 12.09, 11.29 and 9.15 days respectively. Time required to kill 50 per cent of the test population was reduced to a greater extent in combination treatments of the same with Delfin with a range of 5.59 (Delfin+hexane) to 7.77 days (Delfin+acetone) exhibiting temporal synergism (Table 38).

Interaction effect of Delfin with different solvent extracts of *V. negundo* are presented in Table 39. Combination treatments (Delfin+methanol extract, Delfin+hexane extract and Delfin +water extract) resulted in supplemental synergism. Treatment combinations of Delfin and acetone extract exhibited sub additive synergism. Mortality of the larvae caused due to the treatments was 75.87, 89.66, 65.52 and 65.52 per cent (Delfin + methanol, hexane, acetone and water extracts) respectively and were more than the algebraic sum of the mortality caused individually by Delfin and different solvent extracts except acetone viz., methanol, hexane and water extracts resulting 62.4, 74.3 and 62.39 per cent mortality. Treatment combination of Delfin with acetone extract recorded 66.67 per cent mortality and this combined mortality was less than the algebraic sum of the mortality caused individually by Delfin and acetone extracts resulting in 70.25 per cent mortality. Therefore it could be included in sub additive synergism (Table39).

All the treatment combinations of Delfin with methanol, hexane , acetone and water recorded synergestic coefficients of –0.013, -0.015 and –0.015 respectively, indicating sub multiplicative synergism.

4.5.2.4. Combination of Nuclear polyhedrosis virus (NPV) and solvent extracts of V. negundo against S. litura

Results on the combined efficacy of NPV are presented in Tables 40 and 41. Highest mortality of 100 per cent was observed in treatment combination of NPV with methanol extract, while in case of treatment with NPV alone, mortality was only 60 per cent (Plates 13e & 13b). All the combination treatments recorded higher mortalities than the treatment with NPV alone. Treatment combination of NPV with water extract resulted in higher mortality of 86.67 per cent (Plate 13d)

and it was followed by the treatment with NPV and hexane extract with 70 per cent mortality (Plate 13f). Treatment combination of NPV and acetone extract also recorded a comparatively higher mortality of 63.33 per cent when compared to the treatment with NPV alone (Plate 13c). All the solvent extracts of *V. negundo*, except methanol extract recorded very low mortalities when compared to the combination treatments. Hexane extract alone recorded only 40 per cent mortality, but in combination treatment, the same solvent extract recorded a higher mortality of 70 per cent. Similarly acetone extract of *V. negundo* recorded only 43.33 per cent mortality when used alone, while in case of combination treatment (NPV and acetone extract), mortality was increased upto 63.33 per cent. Water extract when used alone resulted in 63.33 per cent mortality, and in combination treatment (NPV+ water extract), mortality rate was increased upto 86.67 per cent.

Lowest time mortality response was observed in treatment combinations of NPV with methanol extract with an LT_{50} of 2.58 days. All the combination treatments of NPV with different solvent extracts of *V. negundo* resulted in very low LT_{50} of 6.76 (NPV+ water extract), 7.52 (NPV+ hexane extracts), and 8.15 (NPV+acetone extracts) days respectively. Treatment with NPV alone took 8.18 days for causing 50 per cent mortality and was on par with the treatment combination of NPV with acetone extract. All solvent extracts of *V. negundo* except methanol extract recorded higher LT_{50} when compared to the combination treatments. Hexane extract when used alone recorded 12.09 days for killing 50 per cent population of test insects, while in combination treatment LT_{50} was reduced to 7.44 days. Similarly, acetone extract when used alone recorded an LT_{50} of 11.29 days and it was lowered to 8.15 days in combination treatment (NPV+acetone). Same trend was also observed in case of water extracts with an LT_{50} of 9.15 days when used alone and there was a reduction upto 6.76 days when the same was used in combination with NPV (Table 40).

Interaction effect of NPV and different solvent extracts are presented in Table 40. All the treatment combinations (NPV+ methanol extract, NPV + hexane extract, NPV+ acetone extract and NPV + water extract) produced suppplimental

Table 40. Mortality of third instar larvae of *S. litura* treated with *V. negundo* extracts in combination with Nuclear polyhedrosis viruses (NPV)

Treatments	Dose (%)	Days after treatment										LT_{50}
		1	2	3	4	5	6	7	8	9	10	
Methanol extract	5	*20	20	43.33	43.33	63.33	73.33	73.33	73.33	73.33	73.33	4.65
Hexane extract	5	0	0	0	0	0	0	20	40	40	40	12.09
Acetone extract	5	0	0	10	10	10	20	30	30	43.33	43.33	11.29
Water extract	5	0	0	0	0	0	0	20	20	53.33	63.33	9.15
NPV	$1.5*10^{12}$ spores/ml	6.67	6.67	13.33	13.33	36.67	36.67	43.33	50	53.33	60	8.18
NPV+methanol extract	$0.75*10^{12}$spores/ml+ 2.5	26.67	43.33	76.67	86.67	100	100	100	100	100	100	2.58
NPV+hexane extract	$0.75*10^{12}$spores/ml+ 2.5	0	0	10	16.67	30	30	46.67	56.67	70	70	7.52
NPV+acetone extract	$0.75*10^{12}$spores/ml+ 2.5	0	0	10	10	20	20	40	53.33	63.33	63.33	8.15
NPV +water extract	$0.75*10^{12}$spores/ml+ 2.5	0	0	0	0	50	50	63.33	63.33	73.33	86.67	6.76

* per cent mortality

Table 41. Effect of combination of Nuclear Polyhedrosis Viruses (NPV) and different solvent extracts of *V. negundo* against *S. litura*

Botanicals	Mortality (%)			Effect	Synergestic coefficient and effect
	M_b	M_v	$M_b + M_v$		
Methanol	73.33 (17.14)	60 (30.88)	100	Supplimental synergism	-0.008 (SMS)
Hexane	40 (29.30)		70	Supplimental synergism	-0.013 (SMS)
Acetone	43.33 (25)		63.33	Supplimental synergism	-0.015 (SMS)
Water	63.33 (17.39)		86.67	Supplimental synergism	-0.009 (SMS)

Figures in paranthesis are theoretical mortality at half dose of fungus and botanicals.

SMS- Sub Multiplicative Synergism

M_b – Mortality (%) caused by *V. negundo* extracts

M_f - Mortality (%) caused by NPV (Nuclear Polyhedrosis Viruses)

synergism. The synergestic coefficients recorded in the treatments were –0.0075, -0.0125, -0.015 and –0.009 indicating sub multiplicative synergism (Table 41).

4.5.2.5. Combination of M. anisopliae and different solvent extracts of V. negundo against H. vigintioctopunctata

Highest mortality of 82.76 per cent was observed in the treatment with hexane extract and was then followed by the treatment in which *M. anisopliae* applied alone (68.97%). In this treatment, upto 8th day of treatment , mortality was very less with only less than 50 per cent mortality (36.67 %), while at the same time, all the combinations of *M. anisopliae* with different solvent extracts of *V. negundo* recorded higher mortalities of 62.07 (*M. anisopliae*+ hexane and *M. anisopliae*+ acetone), 55.18 (*M. anisopliae* + water) and 42.28 (*M. anisopliae* + methanol) per cent respectively.

Acetone extract when treated alone caused only 44.83 per cent mortality, but in combination with *M. anisopliae*, mortality increased upto 62.07 per cent. In case of methanol extract, when treated alone caused 62.07 per cent mortality and was reduced to 42.28 per cent when used in combination with *M. anisopliae* (Table 42).

Time mortality response studies revealed that, the fungus *M. anisopliae* recorded an LT_{50} of 8.2 days when used alone, while in combination treatments except with methanol recorded lower LT_{50} values. Treatment combination of *M. anisopliae* with hexane resulted in lower LT_{50} of 6.32 days and it was then followed by the combination treatment of the same fungus with water and here the LT_{50} recorded 7.44 days and was on par with the treatment with acetone with an LT_{50} of 7.72 days. Even though there was not much variation in the mortalities of the fungus alone and combination treatments, time required to kill 50 per cent population of the test insect is lowered in case of treatment combinations (*M. anisopliae*+ hexane, *M. anisopliae*+ water and *M. anisopliae*+ water) indicating temporal synergism (Table 42).

Interaction effects are given in Table 43. Treatment combinations of *M. anisopliae* with hexane and acetone resulted in sub additive synergism with

Table 42. Mortality of third instar grubs of *H. vigintioctopunctata* treated with *V. negundo* extracts in combination with *M. anisopliae*

Treatments	Dose (%)	Days after treatment										LT_{50}
		1	2	3	4	5	6	7	8	9	10	
Methanol extract	5	*20	20	43.33	43.33	63.33	73.33	73.33	73.33	73.33	*62.07	6.13
Hexane extract	5	0	0	0	0	0	0	20	40	40	82.76	4.38
Acetone extract	5	0	0	10	10	10	20	30	30	43.33	44.83	10.85
Water extract	5	0	0	0	0	0	0	20	20	53.33	55.18	9.09
M. anisopliae	$1*10^8$ spores/ml	0	3.33	13.33	13.33	20	30	33.33	36.67	70	68.97	8.2
M.anisopliae+methanol extract	$0.5*10^8$spores/ml+ 2.5	0	10	6.67	13.33	23.33	43.33	43.33	43.33	50	48.28	9.56
M.anisopliae+hexane extract	$0.5*10^8$spores/ml+ 2.5	13.33	26.67	30	40	40	56.67	56.67	63.33	63.33	62.07	6.32
M.anisopliae+acetone extract	$0.5*10^8$spores/ml+ 2.5	0	10	20	23.33	30	40	53.33	53.33	53.33	62.07	6.32
M.anisopliae+water extract	$0.5*10^8$spores/ml+ 2.5	0	10	26.67	36.67	43.33	46.67	56.67	56.67	56.67	55.18	7.44

* per cent mortality

Table 43. Effect of combination of *M. anisopliae* and different solvent extracts of *V. negundo* against *H. vigintioctopunctata*

Botanicals	*Corrected percentage mortality			Effect	Synergestic coefficient and effect
	M_b	M_f	$M_b + M_f$		
Methanol	62.07 (29.05)	68.97 (45)	42.28	No synergism	
Hexane	82.76 (27.18)		62.07	Sub additive synergism	-0.0072 (SMS)
Acetone	44.83 (35.00)		62.07	Sub additive synergism	-0.010(SMS)
Water	55.18 (33.52)		55.18	No synergism	

* Corrected mortality using Abbot's formula

Figures in paranthesis are theoretical mortality at half dose of fungus and botanicals.

SMS- Sub Multiplicative Synergism

M_b – Mortality (%) caused by *V. negundo* extracts

M_f - Mortality (%) caused by fungus (*M. anisopliae*)

Table 44. Mortality of third instar grubs of *H. vigintioctopunctata* treated with *V. negundo* extracts in combination with *B. bassiana*

Treatments	Dose (%)	Days after treatment										LT_{50}
		1	2	3	4	5	6	7	8	9	10	
Methanol extract	5	*13.33	30	33.33	43.33	46.67	53.33	60	60	63.33	63.33	6.13
Hexane extract	5	30	36.67	43.33	53.33	53.33	60	60	66.67	73.33	83.33	4.38
Acetone extract	5	10	13.33	13.33	13.33	20	30	36.67	40	40	46.67	10.85
Water extract	5	16.67	30	30	33.33	36.67	40	50	50	53.33	56.67	9.09
Beaveria bassiana	$1*10^8$ spores/ml	0	0	0	6.67	6.67	10	16.67	43.33	60	76.67	8.89
B. bassiana +methanol extract	$0.5*10^8$spores/ ml+ 2.5	0	6.67	13.33	13.33	20	23.33	20	36.67	40	43.33	11.19
B.bassiana+hexane extract	$0.5*10^8$spores/ ml+ 2.5	6.67	13.33	26.67	30	56.67	56.67	56.67	63.33	63.33	63.33	6.42
B.bassiana+acetone extract	$0.5*10^8$spores/ ml+ 2.5	6.67	6.67	6.67	16.67	26.67	26.67	40	56.67	56.67	60	8.20
B. bassiana+water extract	$0.5*10^8$spores/ ml+ 2.5	0	10	20	20	30	46.67	56.67	56.67	60	60	7.26

* per cent mortality

Table 45. Effect of combination of *B. bassiana* and different solvent extracts of *V. negundo* against *H. vigintioctopunctata*

Botanicals	Mortality (%)			Effect	Synergestic coefficient and effect
	M_b	M_f	$M_b + M_f$		
Methanol	63.33 (29.05)	76.67 (45)	43.33	No synergism	
Hexane	83.33 (27.18)		63.33	Sub additive synergism	-0.0072 (SMS)
Acetone	46.67 (35.00)		60	Sub additive synergism	-0.013 (SMS)
Water	56.67 (33.52)		60	Sub additive synergism	-0.012 (SMS)

Figures in paranthesis are theoretical mortality at half dose of fungus and botanicals.

SMS- Sub Multiplicative Synergism

M_b – Mortality (%) caused by *V. negundo* extracts

M_f - Mortality (%) caused by fungus (*B. bassiana*)

synergestic coefficients of –0.0072 and -0.010 indicating sub multiplicative synergism (Table 43).

4.5.2.6. Combination of B. bassiana and different solvent extracts of V. negundo against H. vigintioctopunctata

An experiment was conducted in order to test the efficacy of the fungus *B. bassiana* when used in combination with *V. negundo* solvent extracts against *H. vigintioctopunctata* Results are presented in Tables 44 and 45. Highest mortality of 83.33 percent was observed when the grubs were treated with hexane extract alone. Lowest mortality of 43.33 per cent observed in the combination treatment of *B. bassiana* with methanol extract. Methanol extract alone caused a mortality 63.33 per cent and was reduced to 43.33 per cent in combination treatment. *B. bassiana* when used alone produced a mean mortality of 76.67 percent on the tenth day after treatment. From the table it is clear that, in this treatment , mortality started only from the fourth day onwards. Upto 8 th day after treatment, mortality was only 43.33 per cent and was very low when compared to the combination treatments of the fungus with hexane, acetone and water extracts with mean mortalities of 63.33, 56.67 and 60 per cent respectively. Treatment with acetone extract alone recorded only 46.67 per cent mortality and was increased upto 63.33 per cent when used in combination with the fungus *B. bassiana* (Table 44).

Number of days required to kill 50 per cent of the population of test insects were higher in treatment with acetone extract alone with LT_{50} of 10.85 days. When the fungus was applied alone, it took 8.2 days to kill 50 per cent population of the test insects, while in case of combination treatments (*B.bassiana*+ hexane, *B.bassiana* +water and *B.bassiana* + acetone) the LT_{50} values were reduced to 6.42, 7.26 and 8.20 days respectively, indicating temporal synergism in these treatments.

Treatment combinations of *B.bassiana* with hexane, acetone and water recorded sub additive synergism with synergestic coefficients of –0.0072, -0.013 and –0.012 indicating sub multiplicative synergism (Table 45).

Discussion

5. DISCUSSION

5.1. SCREENING OF DIFFERENT PLANT PARTS OF *VITEX NEGUNDO* FOR INSECTICIDAL ACTIVITY

Plant parts of *V. negundo* as leaves, shoots and flowers were tested separately to find out the most potent plant part for insecticidal activity against *Spodoptera litura* Fab. and *Henosepilachna vigintioctopunctata* Fab.

5.1.1. Efficacy of different plant parts and solvents against *S. litura*

Among the different parts (leaves, flowers and shoots) of *V. negundo*, the leaf extracts showed highest mortality (69.17 %) to *S. litura*.

Leaves of *V. negundo* showed significantly highest insecticidal activity (69.17%) while flowers and shoots caused significantly lower mortality in *S. litura* (Fig.1). Leaf extracts with hexane, acetone, water and methanol caused highest mortality (6.67 to 69.17 %) followed by flower (1.67 to 5.83 %) and shoot extracts (0.83 to 5.00 %). Highest potency of leaf extract of *V. negundo* is thus indicated. Among the different solvent extracts tested, methanol and hexane extracts resulted in greater mortalities (86.67 and 80% respectively) (Fig.2). Greater larval mortality has also been reported for *Plutella xylostella* exposed to methanol leaf extracts of syringa (*Melia azaderach*) (Steets, 1975). High larval mortality of methanolic leaf extract of sentang (*Azadirachta excelsa*) was observed against *Plutella xylostella* (Ng *et al*., 2005). They reported that methanolic (sentang) wood extracts caused highest mortality (> 90 %) against *S. litura*. Experiments conducted by Rathi and Gopalakrishnan, (2005) reported that among the different solvent extracts of *Synedrella nodiflora* Gaertn, methanol extracts (0.08 %) resulted in cent per cent mortality when tested against *S. litura*.

The present study revealed that leaf extracts are the most toxic and bio-effective, when compared to shoot and flower extracts and it might be due to the presence of more insecticidal principles in leaf as compared to stem and flowers and hence mode of action will be more faster. Leaf contains more essential oil

Fig. 1

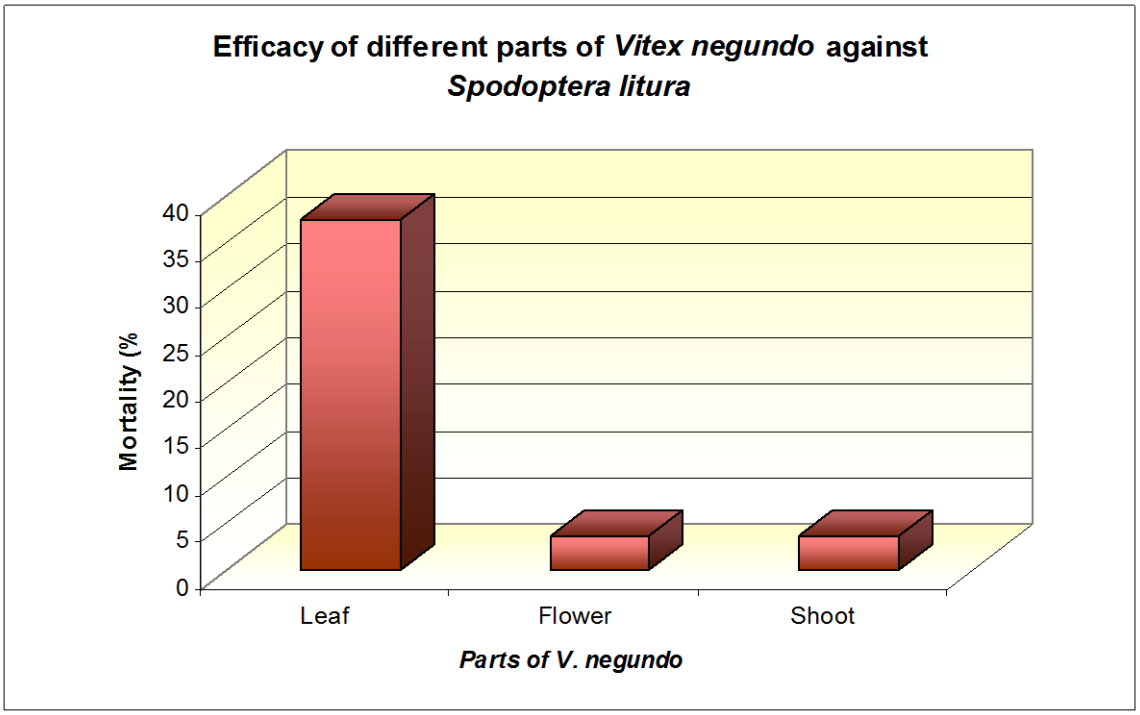
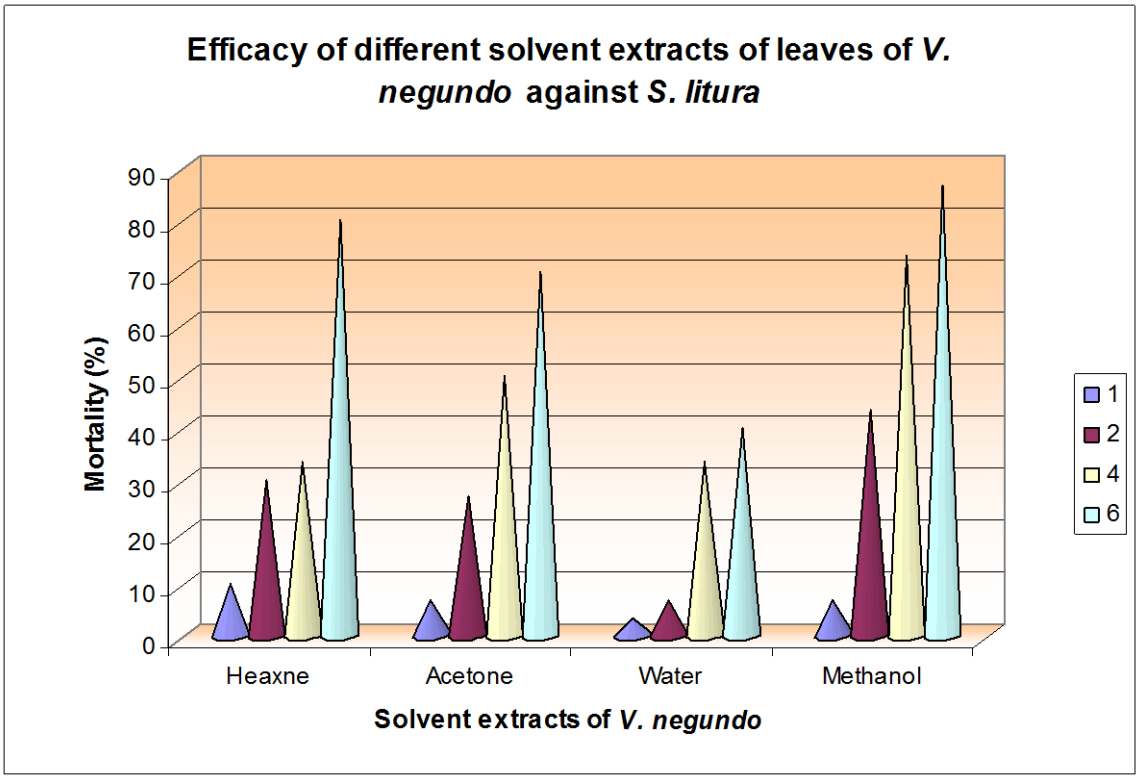

Efficacy of different parts of Vitex negundo against Spodoptera litura
Mortality (%
40
35
30
25
20
15
10
5
0
Leaf
Flower
Shoot
Parts of V. negundo

Fig. 2


Efficacy of different solvent extracts of leaves of V. negundo against S. litura
Mortality (%)
90
80
70
60
50
40
30
20
10
0
Heaxne
Acetone
Water
Methanol
Solvent extracts of V. negundo
1
2
4
6

than stem and flowers indicating its high toxic components and insecticidal action. Among the different solvent extracts, both hexane and methanol recorded highest mortality to *S. litura* and *H. vigintioctapuntata*. Hexane extract which is non- polar in nature may contain more non-polar triterpenoids which has insecticidal action. This is in confirmation with the findings of Othira *et al*., 2009 in which percentage mortality to *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Motschulsky) was highest when treated with hexane extracts of *Hyptis spicigera* leaves.

Study conducted by Rathi and Gopalakrishnan, (2005) revealed that methanol extract of *Synedrella nodiflora* Gaertin was the most effective and toxic among all the tested extracts. Methanol extract resulted to 100 per cent larval mortality of *S. litura*. Preliminary phytochemical analysis of the various extracts of *S. nodiflora* showed that the methanol extract contains steroids, reducing sugars, phenolic compounds, saponins and tannins. Impact of phenolic compounds on the larvicidal activity has been reported by Tripathi *et al*. (2001). In addition to phenolic compounds, tannins reduce the damage of *Maruca testulalis* Geyer (Veeranna, 1992). Steroids also act as insect growth regulators (Wessner *et al*., 1992).

Organic extracts from the leaves of *Vitex mollis* were assessed for their toxic effect on fall armyworm neonate larvae (*Spodoptera frugiperda*). These extracts showed insecticidal and insect growth regulatory activity and Chloroform – Methanol (1:1) extract was the most active (Lopez *et al*., 2007). The difference observed in mortality due to extracts from different plant parts might be because of the variation in the concentration of the chemical constituents in different plant parts. Efficacy of different plant parts and solvent extracts of *Withania somnifera* were tested against *Callosobruchus chinensis* L. by Gupta and Srivastava (2008). Results shown that maximum mortality was observed in insects treated with 10 per cent ether extracts of roots and minimum mortality was found in insects treated with one per cent aqueous extracts of stem and fruits.

In the present study, hexane and methanol extracts resulted in higher mortality followed by acetone and aqueous extracts suggesting that solvent plays an important role in dissolving the plant constituents. The mortality was also found to be concentration dependent with highest at six per cent. An experiment conducted by Srivastava and Mann (1998) using extracts of *Peganum harmala* against *C. chinensis* reported that ether extract at 10 per cent was most effective in causing mortality.

5.1.2. Efficacy of different plant parts and solvents against *H. vigintioctopunctata*

H. vigintioctopunctata, a member from Coleoptera, belonging to the family Coccinellidae was also selected as a test insect in order to find out the difference in the mode of action of plant extract in different insects.

Extracts of different plant parts (leaves, flowers and shoots) were screened for their bioactivity against *H. vigintioctopunctata*. From the Fig.3, it is clear that the leaf extracts were superior than the flower and shoot extracts, resulting in higher mortalities. Leaf extracts recorded significantly higher mortalities ranging from 13.30 to 73.30 per cent, where as flower and shoot extracts caused lower mortalities ranging from 1.667 to 5.830 and 0.883 to 5.000 per cent respectively. The superiority of insecticidal activity of leaf extracts of *V. negundo* is thus indicated against *H. vigintioctopunctata* also (Fig.3 and Table2). Leaf extracts with hexane at six per cent concentration caused highest mortality (86.67 %) and at the same level, lowest mortality was recorded by water extracts (60.00 %) (Fig.4). This is in confirmation with the findings of Othira *et al*. (2009) where they reported that hexane extracts of *H. spicigera* showed the highest mortality to *S. zeamais* when compared to the water and methanol extracts. This is due to the presence of more non polar essential triterpenoids present in the oil extracts. This is due to the presence of more non polar essential triterpenoids present in the oil extracts. Acetone and methanol extracts of *V. negundo* caused 16.67 to 73.33 and 6.67 to 73.33 per cent mortality respectively at different concentrations and they were on par at six per cent concentration.

Fig. 3

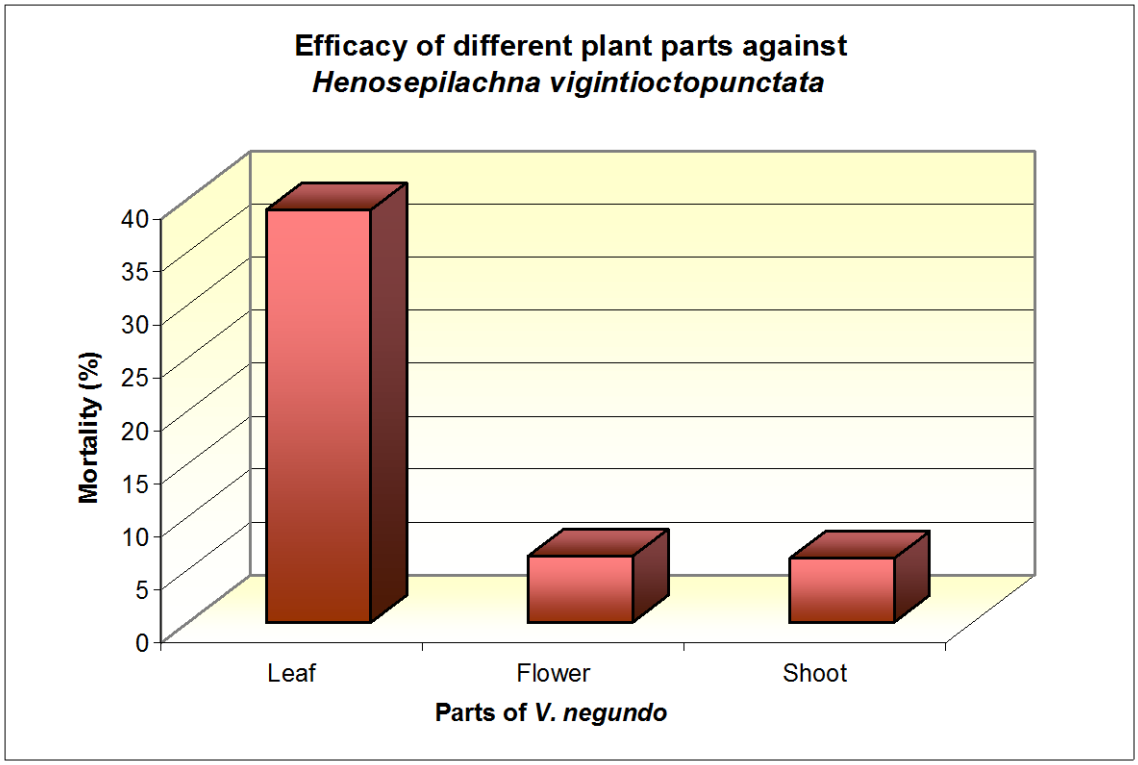
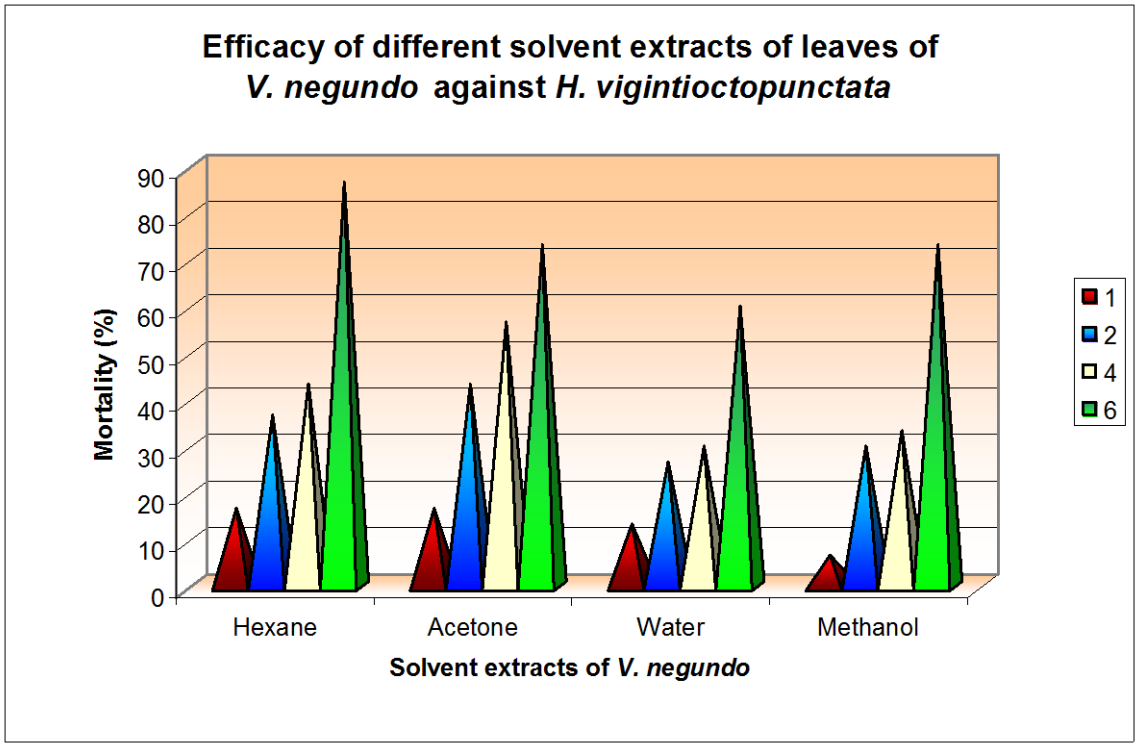

Efficacy of different plant parts against
Henosepilachna vigintioctopunctata
Mortality (%)
0
5
10
15
20
25
30
35
40
Leaf
Flower
Shoot
Parts of V. negundo

Fig. 4


Efficacy of different solvent extracts of leaves of
V. negundo against H. vigintioctopunctata
Mortality (%)
0
10
20
30
40
50
60
70
80
90
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
1
2
4
6

Experiments done by Bing *et al*. (2009) revealed that *V. negundo* essential oil contains 31 compounds of which caryophyllene, eucalyptol and ά- pinene are the main components. Of these the highest toxicity was exhibited my eucalyptol with 100 per cent mortality when tested against *S. zeamais*. All the compounds showed fumigatory effects also and inhibited the activities of AchE (Acetyl choline esterase), CAT (Catalase) and Car E (Carboxyl esterase).

It is thus clear that the leaf extracts are superior in the insecticidal activity as compared to flower and shoot extracts. Hence further studies were conducted with leaf extracts only. A study was conducted in Nigeria by Asawalam *et al*. (2007) to test the insecticidal properties of various plant parts of indigenous plant species against *S. zeamais*. Among the different plant parts tested for their insecticidal activity, seed powder of *Piper guineense* Shum and Thonn, fruit powder of *Capsicum fruitescens* L., leaf powder of *Nicotiana tabacum* L. and seed powder of *Xylopia aetioptica* (Dunal) A. Rich showed significant insecticidal activity.

The present findings suggests that the plant *V. negundo* contains certain chemicals whose content is more in leaves which resulted in the increased mortality of the test insects and therefore act as a potent source for managing the population build up of the most destructive pests *S. ltura* and *H. vigintioctopunctata*. Both the solvents hexane and methanol acted as better solvents than water and acetone for extraction of insecticidal compounds from *V. negundo*. Organic solvents could be better than water to extract metabolites with biological activity.

5.2. MODE OF ACTION OF DIFFERENT EXTRACTS OF *V. NEGUNDO*

5.2.1. Ovipositional deterrent action

5.2.1.1. S. litura

Oviposition of *S. litura* was found to be reduced with increase in concentration from one to six per cent of all the solvent extracts (hexane, acetone, water and methanol) (Fig.5) indicating an inverse relationship between extract concentration and oviposition. The ovipositional deterrent effect of *V. negundo*

was evident from the highest number of eggs laid on leaves treated with the solvents alone in the control treatment.

Methanol extract at six per cent concentration showed highest deterrent effect to oviposition by *S. litura* (Table 3 and Fig 5). This is in confirmation with the findings of Deka *et al*. (1999) who had reported that methanol extracts of *Adathoda vasica* resulted in very few egg laying by *Helopeltis theivora* Waterhouse. Similar results were also observed by methanol extracts of neem at 0.1, 0.15 and 0.2 per cent concentrations against *Helicoverpa armigera* Hubner (Bajpai and Sehgal, 2003). Singh *et al*. (2006) also reported that methanol extracts of ginger resulted in oviposition deterrent activity against *H. armigera*.

All the solvent extracts effectively prevented the adult insects from egg laying with more than 70 per cent oviposition deterrent activity (Fig.5). In controls, where solvents alone were used, hexane and acetone were the highest oviposition deterrents. But among the extracts, methanol extract showed highest deterrency indicating the inherent potency of *V. negundo* as a high oviposition deterrent.

Aqueous extract was the second best ovipositional deterrent with 85.15 per cent reduction in egg laying. Hexane and acetone extracts showed no significant difference in ovipositional deterrency. However they brought about 80 per cent reduction in egg laying. This is in conformity with the findings of Pavunraj *et al*. (2006) who had also observed 80 per cent ovipositional deterrency with hexane extracts of the leaves of *Excoecaria agallocha* (L) against *S. litura*. It was also revealed that aqueous extracts of *V. negundo* at higher concentrations lowered egg laying from 288 at one per cent to 66 at six per cent of *S. litura*. Joshi and Sitaramaiah, (1979) observed that extracts of neem seed kernel (NSKE 2%) deterred *S. litura* adults from egg laying . Acetone extract at six per cent resulted in 80.62 per cent oviposition deterrent activity compared to control treatment. This finding is in agreeable with Subadrabai and Kandaswami (1985) who had reported that acetone extracts of *V. negundo* completely suppressed egg laying of *S. litura*. From the present study it is concluded that among all the four solvent

Fig. 5

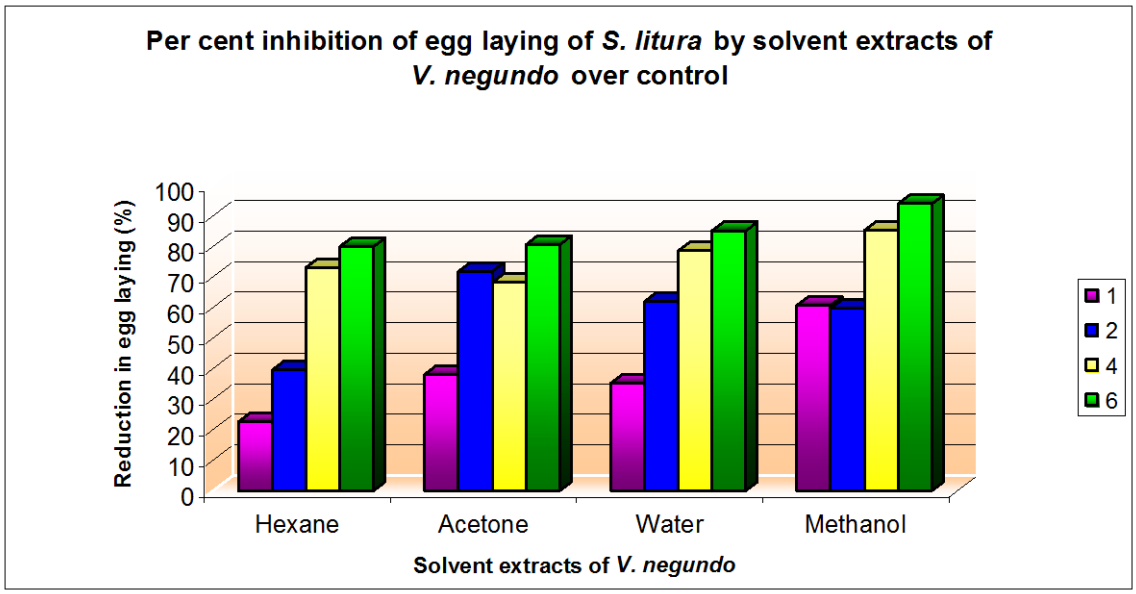
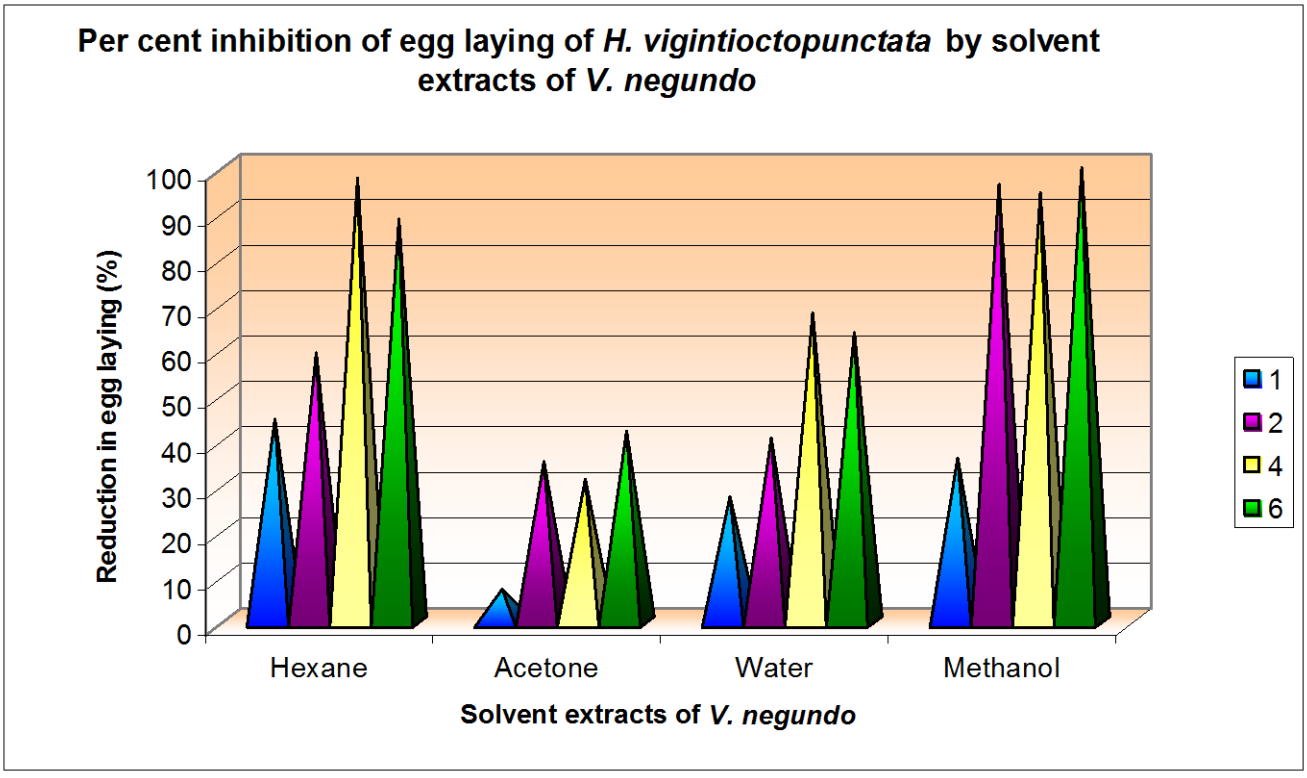

Per cent inhibition of egg laying of S. litura by solvent extracts of V. negundo over control
Reduction in egg laying (%)
100
90
80
70
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
1
2
4
6

Fig. 6


Per cent inhibition of egg laying of H. vigintioctopunctata by solvent extracts of V. negundo
Reduction in egg laying (%)
100
90
80
70
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
1
2
4
6

extracts of *V. negundo*, the solvent methanol at six per cent to have maximum ovipositional deterrency against *S. litura*.

5.2.1.2. H. vigintioctopunctata

Methanol extract (2,4 and 6%) and hexane extract (4%) indicated highest deterrency for oviposition by *H. vigintioctopunctata*. Lowest oviposition deterrent effect was shown by acetone extract (1%). Among all the four solvent extracts acetone extracts showed lowest oviposition deterrent action. Aqueous extracts exhibited a higher deterrency than acetone extracts (Fig.6). Present results are in consonance with the findings of Nair and Thomas (2001), who have found that the aqueous extract of *Acorus calamus* resulted in strong oviposition deterrent action against the melon fly, *Bactrocera cucurbitae.*

The oviposition deterrent effect of *V. negundo* extracts against *H. vigintioctopunctata* was in the descending order of methanol > hexane > water > acetone.

Acetone extract (6%) caused lowest (42.06%) inhibition of egg laying as compared to other solvent extracts. Subadrabai and Kandaswamy (1985) reported that acetone extracts of *V. negundo* when treated on *H. vigintioctopunctata* completely suppressed egg laying. This might be due to the difference in the strain of the test insect as well as the environmental conditions prevailing over here. Rahman and Talukder (2006), reported that among the different plant derivatives tested for their efficacy, nishinda (*V. negundo*) leaf acetone extracts showed highest oviposition deterrent action when tested against pulse beetle *Callosobruchus chinenesis.*

It is thus indicated that among the four solvent extracts, methanol extract was proved to be the most potent inhibitor of oviposition in *S. litura* and *H. vigintioctopunctata*. It caused 94.02 to 100 per cent reduction of egg laying in *S. litura* and *H. vigintioctopunctata* respectively. Hexane, acetone, water and methanol extracts of *V. negundo* caused 80 to 94 per cent inhibition of oviposition in *S. litura*. But the oviposition deterrent action of *V. negundo* extracts ranged from 42 to 100 per cent in *H. vigintioctopunctata*.

5.2.2. Ovicidal activity

5.2.2.1. S. litura

Methanol extracts (6%) caused lowest egg hatching (61.34% inhibition) indicating highest ovicidal action in *S. litura* (Fig.7). This corroborates the findings of Ghatak and Bhusan (1995) who also reported the efficacy of methanol extract of *Annona squamosa* and neem seeds in preventing the emergence of eggs of *Spilosoma obliqua* at two per cent concentration. Ovicidal action of methanol extract of *A. calamus* on *Bactocera cucurbitae* has been well documented by Nair 1996 and Raja *et al*., 2003a. The ovicidal action of hexane extract was on par with acetone extract at four and six per cent. Similarly hexane extract of *Excoecaria agallocha* resulted in ovicidal activity against *S. litura* (Pavunraj *et al*., 2006). Singh *et al*. (2006) also revealed the efficacy of hexane extract of ginger in reducing the hatchability of eggs of *S. litura*.

Aqueous extracts at all the tested concentrations resulted in higher egg hatching and lowest ovicidal action. It caused only 22.32 per cent inhibition of hatching over control at six per cent concentration. Similar results were also observed by Suresh (2002).

It is thus indicated that methanol extract of *V. negundo* at six per cent, caused highest ovicidal action followed by acetone (4 and 6%) and hexane (6%) extract against *S. litura*. This finding is in accordance with the results of Brattsen, (1983) in which he reported that at higher concentrations, neem seed kernel powder (NSKP) reduced egg production and also hatching percentage. Pure triterpenes isolated from *Dysoxylum malabaricum* Bedd. reduced hatching of eggs in rice leaf folder larvae (Nathan *et al*., 2009).

5.2.2.2. H. vigintioctopunctata

A perusal of the data on the hatching percentage of treated eggs of *H. vigintioctopunctata* revealed the ovicidal properties of different solvent extracts of *V. negundo* (Table4 and Fig.8).

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Fig. 7

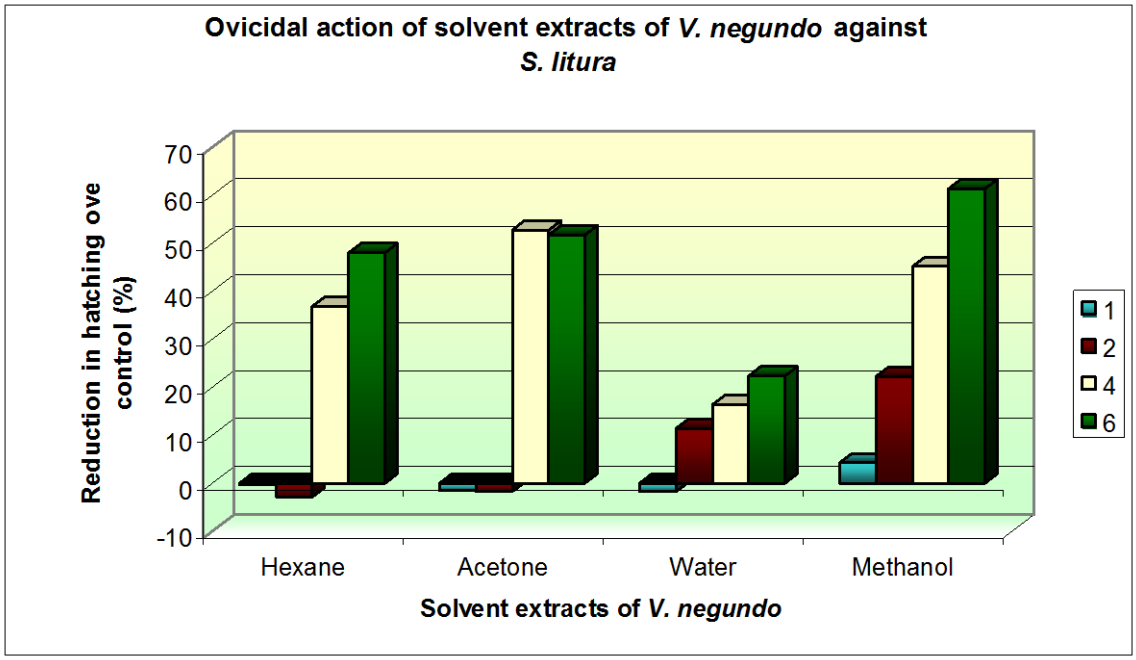
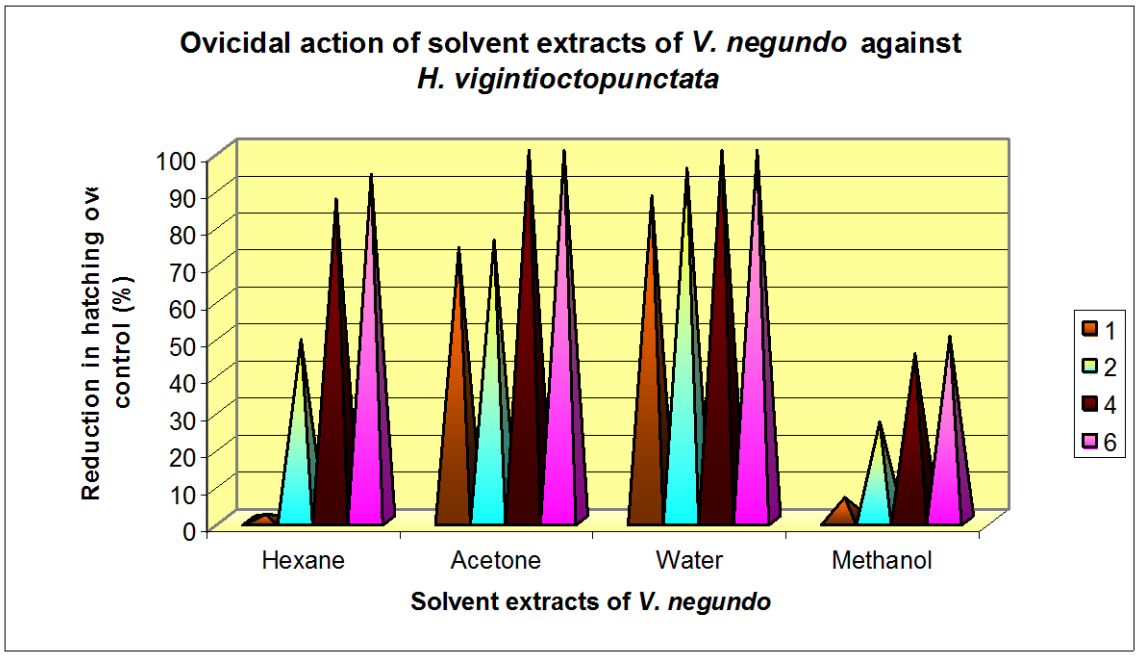

Ovicidal action of solvent extracts of V. negundo against S. litura
Reduction in hatching ove control (%)
70
60
50
40
30
20
10
0
-10
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
1
2
4
6

Fig. 8


Ovicidal action of solvent extracts of V. negundo against H. vigintioctopunctata
Reduction in hatching ov control (%)
100
90
80
70
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
1
2
4
6

No hatching of eggs occured with acetone and aqueous extracts (4 and 6%) This is in confirmation with the findings of Suresh (2002). According to him, *Azadirachta indica* aqueous extracts gave lowest hatching percentage at all the levels (1,5 and 10%).

The ovicidal action of acetone extract was also reported by Saradamma (1989). It was revealed that acetone extracts of *V. negundo, Thevetia nerifolia, A. indica* and *Nerium oleander* when treated on *H. vigintioctopunctata* eggs resulted in cent per cent inhibition from hatching. Acetone extract at lower levels (1 and 2%) also effectively prevented the eggs from hatching with less than 25 per cent hatching. This in confirmation with the findings of Rahman and Talukder (2006) where in highest ovicidal effect of *V. negundo* acetone extracts were reported against *C. chinensis*. Hexane extracts at higher concentrations (4 and 6%) reduced hatching (86.79 to 93.60%) considerably. Methanol extract caused lowest inhibition of hatching (49.76%).

It can be concluded that the solvent extracts with water and acetone at all the levels (1,2,4 and 6%) could inhibit hatching of eggs of *H. vigintioctopunctata*. Higher concentrations (4 and 6%) resulted in cent per cent inhibition from hatching.

5.2.3. Growth and developmental effects

5.2.3.1. Effect of different solvent extracts of V. negundo on pupal weight of S. litura on castor, semi-synthetic diet and banana

When *V. negundo* solvent extracts treated food were fed to *S. litura* at different concentrations (1, 2, 4 & 6%), effects on development were noted. There was a significant variation in the pupal weight of the test insect in different treatments. Reduction of pupal weight with the increase in concentrations of different solvent extracts was observed in *S. litura* reared on different hosts.

S. litura reared on castor revealed that in all the treatments, the pupal weights were lower when compared to control. Increase in concentrations of the extracts caused a decrease in growth and weight of pupae (Fig.9). This reduction in growth could be attributed to two possible modes of action, namely

antifeedancy or chronic toxicity. Further investigations are necessary for conclusive remarks. Nutritional experiments were also carried out to differentiate the behavioural effects and post-ingestive toxicity and to determine the exact mode of action, which is presented later.

All solvent extracts of *V. negundo* caused significant reduction in pupal weight of *S. litura* reared on castor as compared to that in solvents alone. Pupal weight was found to decrease with increase in concentration. Acetone extract of *V. negundo* caused highest reduction (55.25 %) in weight of *S. litura* pupae while hexane, water and methanol extracts showed 45 to 49 per cent reduction in pupal weight (Fig.9).

In case of pupae of *S. litura* reared on semi-synthetic diet, methanol extract caused highest reduction in pupal weight (72.60%) over control while water extract brought about a lowest reduction (56.51%) of pupal weight. Acetone extract also resulted in higher reduction (70%) of pupal weight over control.

When the larvae were reared on banana also, methanol extract resulted in highest reduction in pupal weight (65.62%) followed by hexane extract (39.9%). Water extract again showed lowest (52.91%) pupal weight reduction.

It is thus indicated that out of three food sources, *V. negundo* caused highest reduction (56.5 to 72.60 %) of pupal weight in *S. litura* reared on semi-synthetic diet and among the extracts, methanol extract caused highest reduction (45.15 to 72.60 %) and aqueous extract resulted in lowest reduction (25.91 to 56.51%) of pupal weight of *S. litura* reared on three hosts. The present finding on the reduction of pupal weight in *S. litura* due to *V. negundo* extracts is in conformity with Govindachary *et al*. (1996) who reported decreased pupal weight in *S. litura* due to neem.

5.2.3.2. Effect of different solvent extracts of V. negundo pupation of S. litura on castor, semi-synthetic diet and banana

V. negundo extracts produced significant reduction in the pupation of S. litura reared on all hosts *viz*., castor, semi-synthetic diet and banana. Pupation was reduced with increase in concentration extracts showing lowest pupation with the

Fig. 9

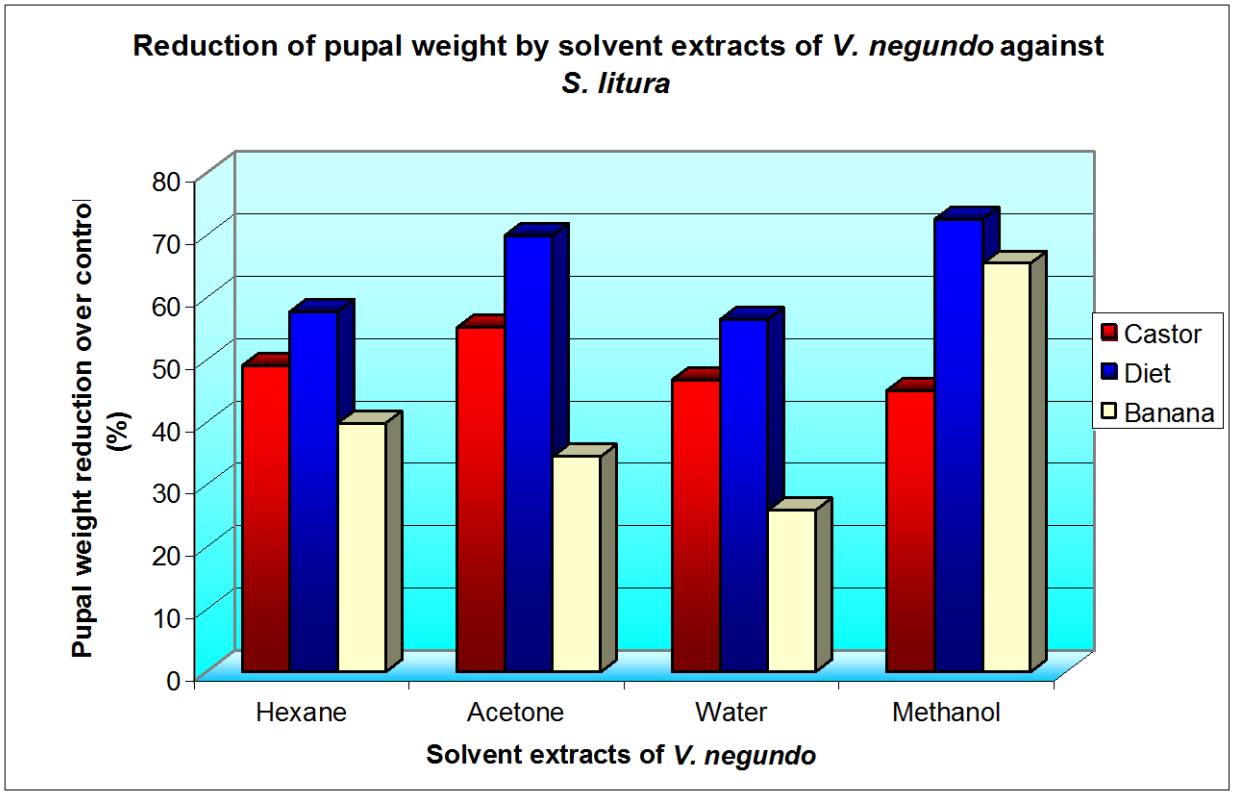
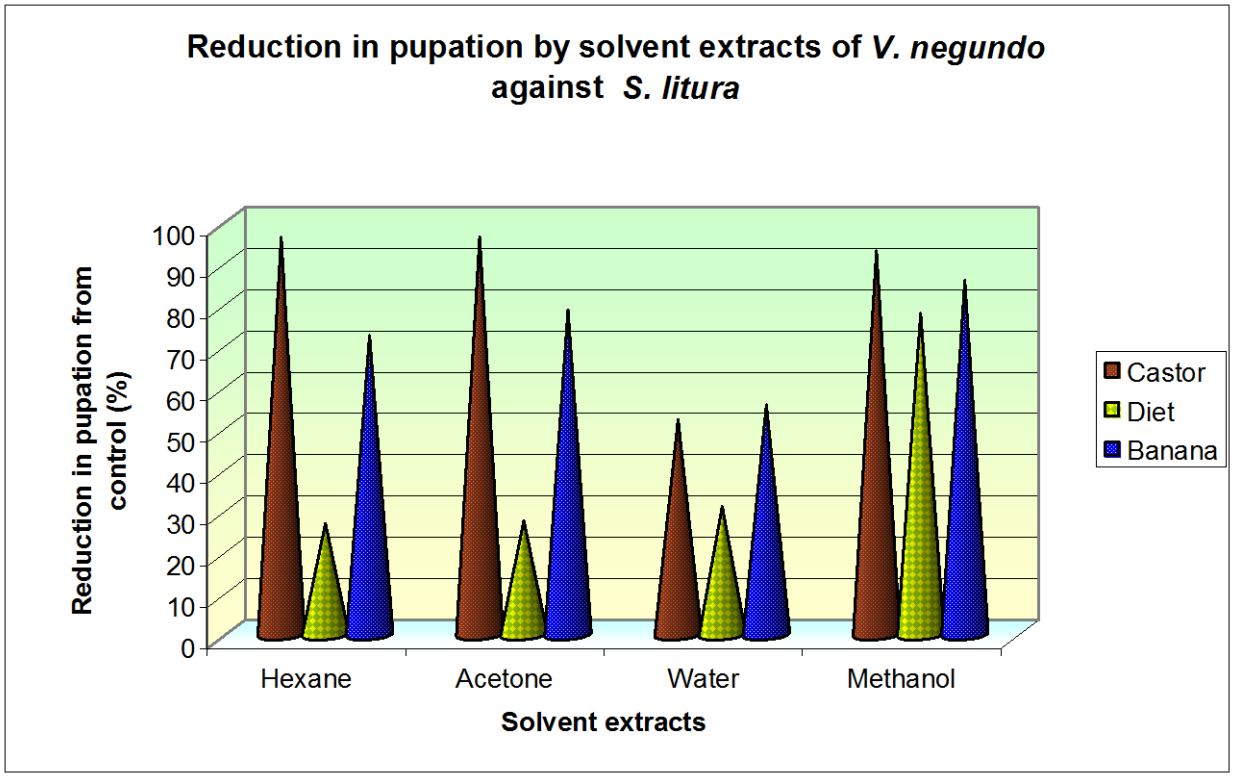

Reduction of pupal weight by solvent extracts of V. negundo against S. litura
Pupal weight reduction over control (%)
0
10
20
30
40
50
60
70
80
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
Castor
Diet
Banana

Fig. 10


Reduction in pupation by solvent extracts of V. negundo against S. litura
Reduction in pupation from control (%)
0
10
20
30
40
50
60
70
80
90
100
Hexane
Acetone
Water
Methanol
Solvent extracts
Castor
Diet
Banana

highest concentration of six per cent in all solvent extracts. *V. negundo* extracts caused lowest pupation (3.33 to 53.33%) on castor and highest (20 to 96.67%) on semi-synthetic diet. Among the four solvent extracts, hexane and acetone extracts caused lowest pupation (3.33%) on castor, but acetone extract (6%) resulted in highest pupation (70%) when *S. litura* was reared on semi-synthetic diet indicating its lower influence on pupation. Aqueous extracts caused 40 to 66.67 per cent pupation (Table 6).

All the solvent extracts of *V. negundo* except water resulted in more than 90 per cent reduction in pupation of *S. litura* reared on castor (Fig.10). Influence of *V. negundo* solvent extracts was comparatively lower in larvae reared on semi-synthetic diet. Here also methanol extract recorded highest reduction in pupation (77.77 %). All the solvent extracts recorded more than 50 per cent reduction in pupation of *S. litura* reared on banana with highest reduction in pupation by methanol extract (85.72 %). Aqueous extract caused 31.03 to 55.56 per cent reduction in pupation while 77.78 to 92.86 per cent reduction was caused by methanol extract thus indicating the significant effect of methanol extract in reducing the pupation of *S. litura*. Pupation rate was significantly reduced when *Pieris rapae* L. larvae were treated with the botanical rhodojaponin III (Zhong *et al*., 2001). Huang *et al*., (2004) reported that azadirachtin at 1 ppm concentration reduced pupation of *S. litura* to 60 per cent.

Results of the present study are in accordance with the findings of Singh *et al*. (2005). They had reported that pupation percentage of *E. vitella* showed a reducing trend with increase in concentration of different solvent extracts of ginger. Benzene fractions of the extracts at two per cent level recorded minimum pupation of only 14.4 per cent.

5.2.3.3. Effect of different solvent extracts of V. negundo larval duration of S. litura on castor, semi-synthetic diet and banana

When the larvae were reared on castor, acetone extract at six per cent resulted in maximum larval duration (62.81%) and was followed by aqueous extract with 85.87 per cent increase in larval duration over control (Fig.11).

Solvent extracts of *V. negundo* caused significant increase in larval period of *S. litura*. Larval duration was increased with increase in extract concentration. Larval period showed variation when *S. litura* were reared on different hosts treated with six per cent *V. negundo* extracts. Larvae on semi-synthetic diet had taken longest period (10.67 to 26.33 days) while it was shortest on banana from 12.67 to 17.67 days (Table7).

Among the four solvent extracts, methanol extract caused longest larval period (15 to 26.33 days) and shortest with water (17.70 to 18.0 days). This is in consonance with the findings of Singh *et al.* (2005). According to them, mean larval period of *E. vitella* prolonged in all fractions of methanol extracts of ginger. Longest duration of 17.3 days (in control, only 9.7 days) was observed in the acetone fraction. Acetone extracts at two per cent concentration also recorded longer larval duration of 21.67 days. Hexane extracts at four per cent level and the aqueous extract at six per cent level also recorded higher larval duration of 19 days and were on par with each other.

Control larvae reared on banana took an average of 14 to 14.66 days for pupation. Here the insects treated with water extracts at six per cent level took more days for pupation. It was immediately followed by hexane extracts at the same level and acetone extracts at four per cent level. Highest duration observed by the methanol extract treatment was 17.67 days. The lowest larval duration of 12.67 days was observed by hexane at one per cent level.

Results of this study are in confirmation with the findings of Martinez and van Emden (2001). According to these scientists, *S. littoralis* third instar larvae when exposed to different concentrations of azadirachtin took longer time to reach the pre-pupa in comparison with the control insects. Similar results relating to the whole larval stage of *S. frugiperda* were obtained by Redfern *et al*. (1981) and *S. litura* by Behera and Satapathy (1997). Larvae kept on treated diet had their pupal ecdysis delayed in comparison with the control insects. Essential oil of *Vitex trifolia* and *Vitex aganus-castus* were evaluated against fifth instar larvae of *Spilosoma obliqua*. This treatment caused extended larval and pupal period (Tandon *et al*., 2008). Prolonged larval duration with reduced weight was

Fig. 11

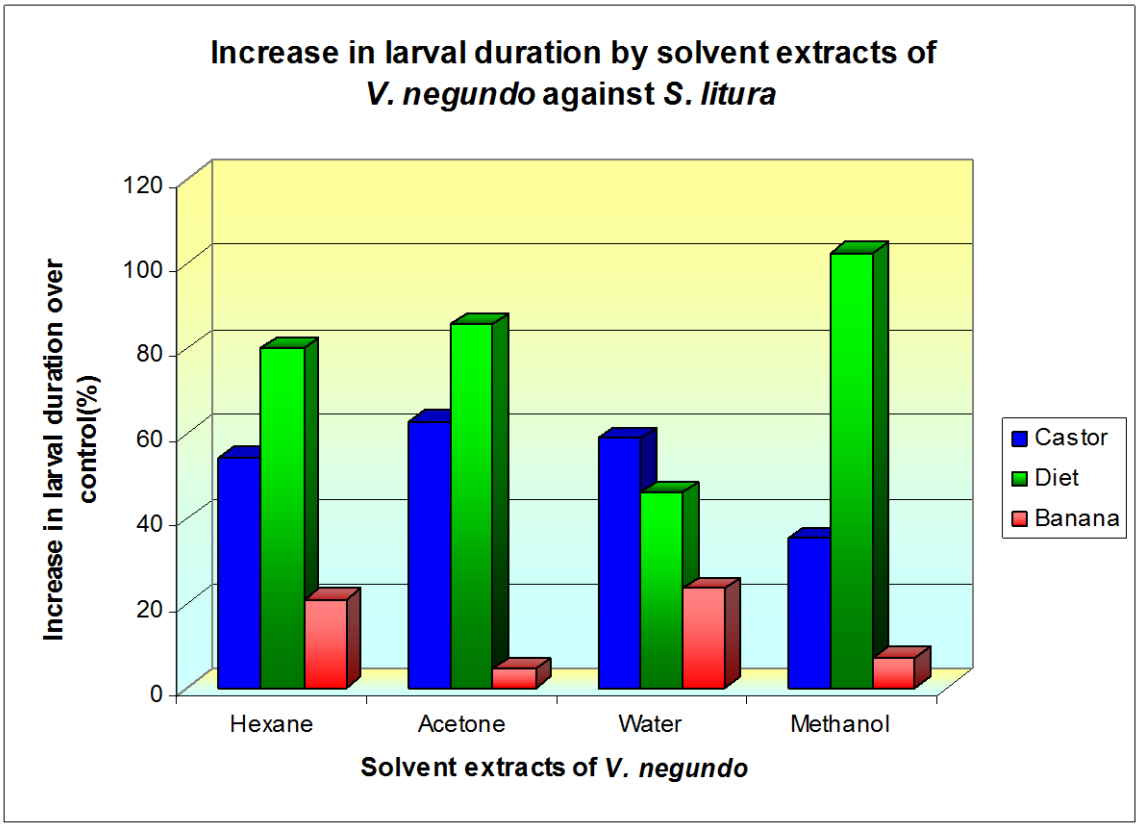

Increase in larval duration by solvent extracts of V. negundo against S. litura
Increase in larval duration over control(%)
120
100
80
60
40
20
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
Castor
Diet
Banana

Fig. 12

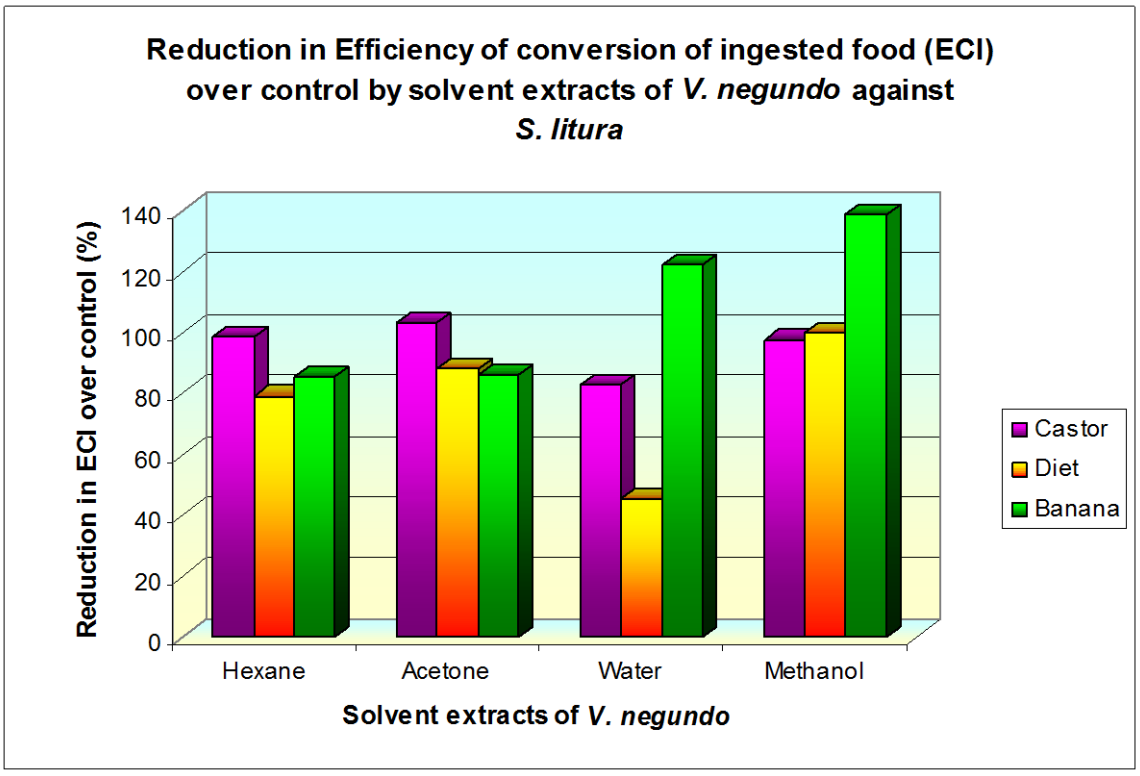

Reduction in Efficiency of conversion of ingested food (ECI) over control by solvent extracts of V. negundo against S. litura
Reduction in ECI over control (%)
140
120
100
80
60
40
20
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
Castor
Diet
Banana

Fig. 13

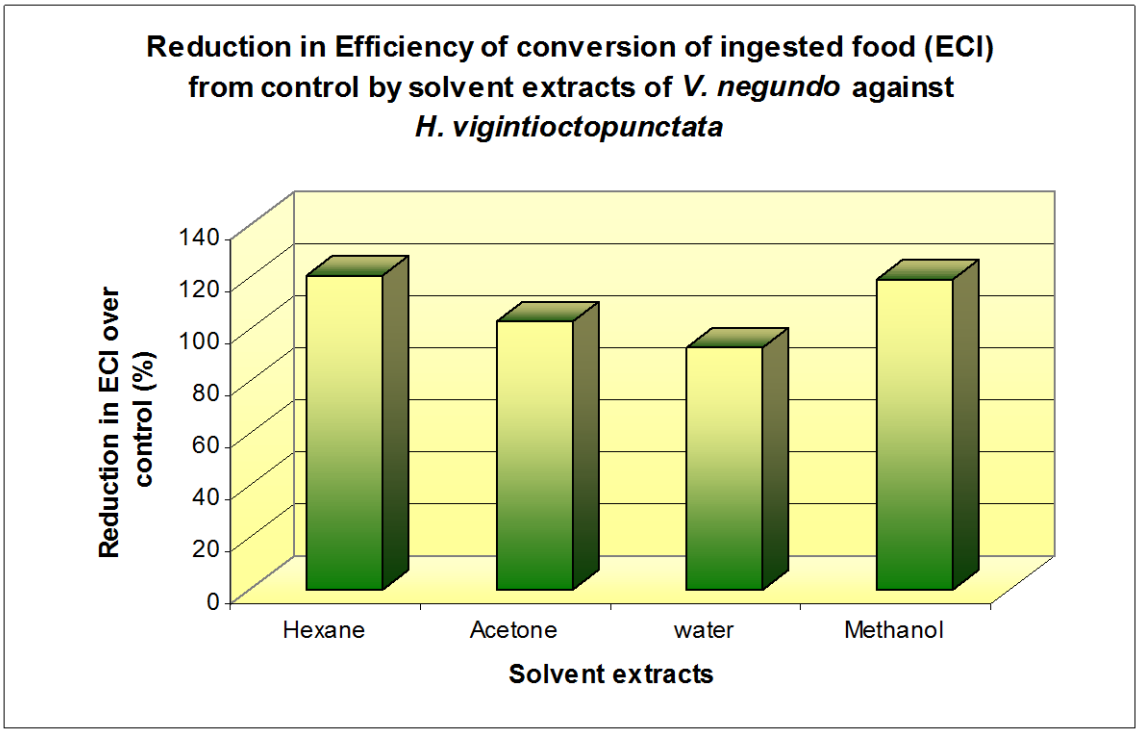
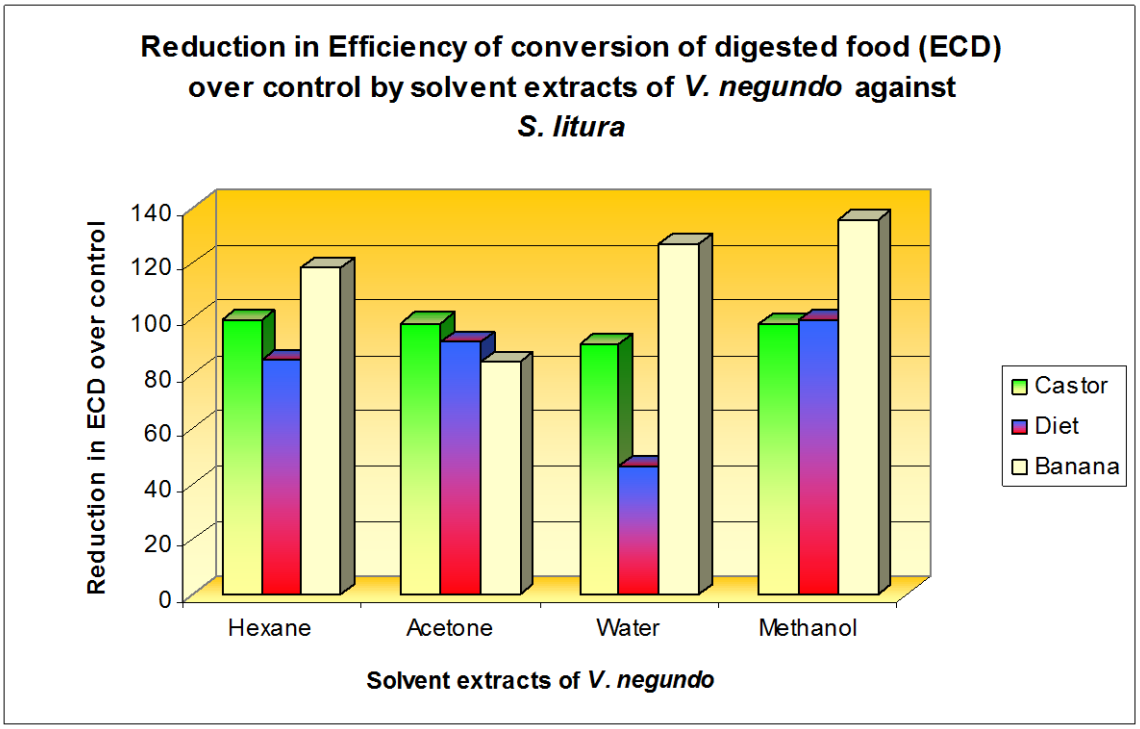

Reduction in Efficiency of conversion of ingested food (ECI) from control by solvent extracts of V. negundo against H. vigintioctopunctata
Reduction in ECI over control (%)
140
120
100
80
60
40
20
0
Hexane
Acetone
water
Methanol
Solvent extracts

Fig. 14


Reduction in Efficiency of conversion of digested food (ECD) over control by solvent extracts of V. negundo against S. litura
Reduction in ECD over control
140
120
100
80
60
40
20
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
Castor
Diet
Banana

observed when larvae of rice leaf folder were treated with pure triterpenes isolated from the plant *Dysoxylum malabaricum* Bedd. (Nathan *et al*., 2009).

5.2.4. Antifeedant action

5.2.4.1. Effect of different solvent extracts of V. negundo on the percentage of leaf protection against S. litura on castor, semi-synthetic diet and banana

Data relating to the percentage of leaf protection are given in Table 8. On castor, highest feeding inhibition observed in treatment with aqueous extract at six per cent concentration with a mean percentage leaf protection of 22.619. This is in agreement with Sahayaraj (1998) who reported that, highest antifeedancy was shown by *V. negundo* acqueous extracts against *S. litura* when compared with other organic solvent extracts. At the same, it was found that acetone extract of the same showed good leaf protection/antifeedancy against *Earias vitella, Diaphania indica* and *Epilachna septima* (Kalavathi *et al*., 1991). From the above reports it is clear that there is conflicting effects of the same botanical with different solvent extracts on different test insects. Hence, the selection of botanical, method of extraction, target insect, etc. have to be taken care of when they are recommended for IPM programmes.

In the present study, none of the treatments recorded more than 50 per cent leaf protection. Even at higher concentrations (6%) the solvent extracts of acetone and methanol recorded very low feeding inhibition of -5.159 and -5.357 per cent (negative value was due to higher consumption in the treatment as compared to control). The lowest feeding inhibition of -15.099 per cent was observed by acetone extracts at four per cent concentration and it was then followed by water extracts at one per cent concentration with -13.095 per cent. This is in confirmation with the findings of Saradamma (1989) and Suresh (2002) who also had reported that the acetone extract of *V. negundo* gave a low level of leaf protection (36.23 %).

No feeding inhibition was observed in semi-synthetic diet. All treatments recorded lowest feeding inhibition except extracts with water at four per cent concentration, which recorded mean leaf protection of 25.563 per cent.

The experiment when conducted on banana resulted in the following findings. Solvent extracts of hexane, even at higher concentrations resulted in lowest antifeedant action, whereas methanol extracts even though recorded poor leaf protection at lower concentrations gave a higher leaf protection of 39.499 per cent at six per cent concentration. Highest leaf protection (42.389%) was recorded by acetone extract at six per cent concentration followed by water extract (41.137%). None of the treatments resulted in more than 50 per cent deterrency. This is in contradiction with the findings of Sharma *et al*. (2001) where he reported 100 per cent deterrency in feeding against third instar larvae of *S. litura* when treated with leaf essential oils of *V. negundo*. Lower feeding deterrency in present finding could be attributed to the lower activity of phytochemicals responsible for antifeedant action in solvent extracts as compared with essential oils.

Among all the solvent extracts, highest leaf protection of 42.39 and 41.14 per cent was achieved by acetone and aqueous extracts at six per cent concentration, which is on par with each other. Irrespective of the hosts on which *S. litura* larvae were reared, *V. negundo* extracts showed no feeding deterrency. Highest antifeedant action was recorded by *V. negundo* extracts of acetone (42.39 %) in case of *S. litura* larvae reared on banana. But that too was below 50 per cent. From this study, it could be concluded that antifeedant action is not the possible mode of action. Because antifeedancy is essentially a preingestive effect, based on chemosensory responses, where the insect rejects food treated with the compound and ingestion is reduced. Here in some of the treatments, the mean percentage leaf protection was negative indicating more consumption by the treated larvae as compared to the control larvae. None of the treatments results in more than 50 per cent leaf protection. Further investigations are essential to find out the other possible modes of actions like chronic toxicity, insect growth regulatory (IGR) effects etc.

5.2.4.2. Effect of different solvent extracts of V. negundo on the percentage of leaf protection of H. vigintioctopunctata

Antifeedant action of *V. negundo* solvent extracts against epilachna beetles were worked out and the results presented in Table 9.

In most of the treatments antifeedancy recorded negative indicating higher consumption in treatments than in the control. Lowest antifeedancy (–36.111 %) was obtained in the treatment with hexane extract at two per cent level. In case of aqueous extract, all the treatments recorded negative values indicating higher consumption in the treatments. Highest antifeedancy was recorded by extracts with acetone at six per cent concentration with a mean value of 22.963 per cent. None of the treatments recorded more than 50 per cent antifeedancy. Solvent extracts of *V. negundo* were not acting as feeding deterrents against *H. vigintioctopuncta*. From this it is clear that antifeedancy is not the mode of action of *V. negundo* extracts. Similar results of no antifeedancy were reported by Singh and Rao (2000) in ten per cent petroleum ether extract of *Ageratum conyzoides*.

5.2.5. Larval starvation

5.2.5.1. Effect of different solvent extracts of V. negundo on percentage larval starvation against S. litura on castor leaf, semi-synthetic diet and banana leaf

Table10 depicts the results of larval starvation as indicated by the differential weights of larvae at different concentrations in comparison with control larvae.

On castor, all the solvent extracts at the highest concentration (6%) caused highest per cent starvation in the larvae of *S. litura*. Among the four types of extracts acetone extract resulted in a significantly highest (51.48%) larval starvation (45%) in *S. litura* larvae. Hexane and methanol extracts showed no significant difference in larval starvation.

Larval starvation was lower in semi-synthetic diet reared *S. litura* than in castor. Methanol extract at six per cent resulted in highest larval starvation

(44.24%). All the other three extracts showed significantly lower starvation to larvae of *S. litura*.

In case of *S. litura* larvae reared on banana, all the four extracts of *V. negundo* caused a similar trend of highest larval starvation at the highest concentration of six per cent. Highest larval starvation (74.29%) was recorded by methanol extract followed by water extract (72.95%). Hexane and acetone extracts also recorded more than 50 per cent larval starvation (52.77 and 54.56% respectively).

5.2.5.2. Effect of different solvent extracts of V. negundo on percentage larval starvation of H. vigintioctopunctata

Findings of the experiment were analysed statistically and the results were presented in Table 11.

Percentage larval starvation of *S. litura* showed an increasing trend with increase in concentrations of *V. negundo* extracts. Highest larval starvation (117.094%) was recorded by the solvent extract with hexane at six per cent level. It was followed by methanol extract at the same level with 101.709 per cent larval starvation. Acetone at all the levels recorded more than 65 per cent larval starvation and were on par with each other. Lowest larval starvation was observed in methanol extracts at one and two per cent levels which was less than 20 per cent. But it has considerably increased upto 101.709 per cent at six per cent level. Aqueous extracts at lower concentrations recorded less than 50 per cent larval starvation, but was increased upto 68.376 per cent at four per cent level.

From this experiment it could be inferred that in both the test insects, the trend is somewhat different which could be attributed to the fact that different insects will respond differently to the same extract.

In case of *S. litura,* the results of the experiment done on the semi-synthetic diet showed that percentage larval starvation was very less when compared to castor and banana. This may be due to the high nutritive quality of the diet when compared to the natural hosts, castor and banana which helped to utilize the ingested food for growth and to over come the xenobiotic effect of the extract to

some extend. Hence larval starvation values are very less. Banana recorded high larval starvation values when compared to castor and semi-synthetic diet.

Results of the experiment done on epilachna showed that even though consumption rate is high (in most cases, negative values indicating higher consumption in the treatments when compared to control), it is not reflected in the larval growth. Energy is utilized for the metabolic activities, to detoxify the xenobiotic compounds present in the extract. Hence percentage larval starvation recorded very high values.

5.2.6. Approximate digestibility (AD)

5.2.6.1. AD as influenced by different solvent extracts of V. negundo against S. litura on castor leaf, semi-synthetic diet and banana leaf

On castor, all the four extracts of *V. negundo* resulted in higher AD values than the control in *S. litura*. Hexane extract caused highest value ranging from 90.6 to 96.52 per cent of AD and methanol extract produced lowest AD values of 61.50 per cent (1%) to 86.06 per cent with six per cent extract (Table12).

V. negundo extract showed the same trend of higher AD values in *S. litura* fed on semi-synthetic diet than those in control. Acetone extract caused highest AD values ranging from 92.63 (2% extract) to 96.56 per cent (6% extract).

When *S. litura* were fed on banana, all the solvent extracts except water and methanol extracts (1%) resulted in lower AD values compared to respective controls. Aqueous extract caused highest AD values ranged from 65.46 to 85.62 per cent. Hexane extract revealed lowest AD values with 28.72 in six per cent extract to 61.70 in four per cent extract.

A comparison of AD values of *S. litura* on three hosts has thus indicated highest AD on semi-synthetic diet and lowest on banana. Among the four extracts, acetone extract caused highest AD value and lowest with methanol extract. The present finding on higher AD values in treatments with *V. negundo* might have been due to the changes at the levels of digestive enzymes. Kumar and Ahmed, (2006) reported that AD of *Taragama siva* remained high when fed with higher concentrations of plant extracts which reflects an attempt by the insect to

compensate for reduced consumption and utilization of treated leaves in order to maintain growth. Higher AD values in treatments when compared to control could presumably be attributed to the fact that larvae defaecate excessively the leaves containing toxic substance in a physiological event to remove the toxic substance and hence relatively less material is allocated to body tissue. Similar results were also observed by Fagoonee (1984) in *C. binotalis*. Nathan *et al*., 2004 also reported higher AD in the treated insects (15.25 % more) when compared to control in rice leaf folder larvae treated with *Dysoxylum* triterpenes. They also reported that although the treated larvae were capable of maintaining AD (increased during treatment), they failed to maintain the RGR during development. Significantly increased AD on treated insects was determined by some authors (Meisner *et al*., 1982; Antonius and Hegasy., 1987; Gonzalez *et al*., 1992; Hussain, 2000; Garside *et al*., 2000; Mohamed *et al*., 2003). Increased AD value might be due to the attempts made by the insect to compensate for reduced consumption and utilization of food in order to maintain growth rates (Reese and Beck, 1976).

5.2.6.2. Effect of different solvent extracts of V. negundo on AD of H. vigintioctopunctata

Effects of *V. negundo* extracts on AD in *H. vigintioctopunctata* is presented in Table 13.

All the extracts showed a higher AD values ranging from 98 to 99 per cent as against the control value of 97 to 98 per cent.

5.2.7. Efficiency of conversion of ingested food (ECI)

5.2.7.1. ECI as influenced by different solvent extracts of V. negundo on castor leaf, semi-synthetic diet and banana leaf against S. litura

Results of the experiment are presented in Tables14 and 14a and Fig.12.

All the *V. negundo* extracts caused significantly reduced ECI of *S. litura* reared on castor, diet and banana. ECI values showed a declining trend with increase in concentration of *V. negundo* extract. Similar observations were earlier

reported by Chitra and Rao (1996) where in they had observed that ECI was inversely proportional to concentrations in *S. litura* treated with *A. indica, N. odorum* and *Argemone mexicana* and *V. negundo* extracts.

Experiments done on castor, revealed that in all treatments, ECI values were very low when compared to the control. In all the treatments ECI showed a declining trend with increase in concentration. All the treatments with *V. negundo* extracts recorded very low ECI values when compared to respective controls (solvents alone) indicating the effect of *V. negundo* in reducing ECI of *S. litura* larvae reared on castor.

All solvent extracts recorded the highest ECI (7.35 to 13.34 %) at one per cent concentration showing their assimilatory inhibition even at lower concentrations (Table 14). On castor reared *S. litura* larvae, maximum reduction of ECI (103.46 %) was recorded by acetone extract (6%) followed by hexane and methanol extracts (98.59 and 97.63% respectively). Aqueous extract also resulted in higher reduction (82.85%) in ECI over control (Fig.12). Similar findings were reported by Singh *et al*., (2005) in which they reported that among different fractions of ginger extract, ECI was lowest in two per cent acetone fraction with a mean value of 1.5 per cent. Methanol, hexane and benzene fractions also recorded lower ECI values of 4.48, 5.43 and 3.30 per cent respectively.

In case of diet fed larvae, even though consumption rate was high, the insect was unable to assimilate the ingested food. Here lowest ECI value was obtained by methanol extract at six per cent level (0.061%) proving complete inhibition of assimilation of the ingested food. It was followed by the same solvent at four per cent level (2.600%) proving the growth inhibitory potential of methanol extract. At six per cent level the solvent extracts of acetone and hexane also recorded lower ECI of 3.980 and 5.429 per cent respectively (Table 14). Methanol extract caused highest reduction in ECI (138.69 and 99.73% respectively) in both banana and diet reared *S. litura* larvae. In case of larvae reared on semi-synthetic diet, acetone and hexane extracts also recorded higher

reduction in ECI (88.02 and 79.17 % respectively) while water extract recorded lowest reduction of only 45.35 per cent.

From lower to higher concentrations (1 to 6%), a reducing trend in ECI values was observed in all the solvent extracts of *V. negundo* when the experiment was carried out on banana also. The lowest ECI was observed in methanol extract at six per cent level (-14.81 %) and was followed by water extract at six per cent level and methanol extract at four per cent levels (-11.60 and -9.32 %). The lower ECI might have due to the complete inhibition of assimilation of food by *S. litura*. This result was in agreement with the findings of Sundararaj *et al*. (1995). According to them, amount of food ingested by *T. siva* was least on the host plants treated with 0.05 per cent methanol extracts of Neem Seed Kernel Powder (NSKP) and significantly different from that of acetone and control. On banana reared *S. litura* larvae, aqueous extract resulted significantly higher reduction in ECI (122.40 %) over control. Hexane and acetone extracts also recorded more than 80 per cent reduction of ECI over control (85.23 and 86.29 % respectively) (Fig.12). Sharma *et al*. (1982) observed that differences in food quality were not seen in their digestibility but in their efficiency of digested / ingested food to body substance. Sahayaraj (1998) reported that plant extracts of *A. indica, Zingiber officianale, Citrus sinensis, V. negundo* when treated against *S. litura* resulted in drastic decline of ECI (-53.58, -51.87, -41.49, -47.79 % respectively).

The inhibitory potential of *Dysoxylum* triterpnes in reducing the ECI when tested against rice leaf folder was reported by Nathan *et al*. (2007). ECI is an overall measure of an insect's ability to utilize the food that it ingests for growth. A drop in ECI indicates that more food is being metabolized for energy and less is being converted to body substance (*i.e*. growth). Decrease in ECI values indicate that ingested *V. negundo* extracts does also exhibit some chronic toxicity. Similar results were also seen with *Dysoxylum* extracts (Nathan *et al*., 2007), *Trichilia Americana* extract (Wheeler and Isman, 2001), rotenone (Wheeler *et al*., 2001), chinaberry extract (*M. azaderach* L) (Nathan, 2006) and neem limonoids (Nathan *et al*., 2005) when tested against lepidopteran pests.

5.2.7.2. ECI as influenced by different solvent extracts of V. negundo against H. vigintioctopunctata

The results of the experiment are given in Table 15 and Fig.13.

All extracts of *V. negundo* drastically reduced ECI in *H. vigintioctopunctata* indicating high potency of *V. negundo* extracts. Maximum reduction in ECI (120.87%) was recorded by hexane extract and was followed by methanol and acetone extracts (118.97 and 103.18% respectively). Aqueous extract also resulted in significantly higher reduction in ECI (92.81%) over control (Fig.13).

At lower concentrations it self, all solvent extracts recorded very low ECI values proving their efficiency in preventing assimilation of ingested food (Table15). *V. negundo* extracts with hexane, acetone and methanol recorded negative ECI values in both test insects indicating that they can be used as potential growth inhibitors preventing normal weight gains and consequently growth of the insect. This is in confirmation with the findings of Sahayaraj (1998) where he had reported the efficacy of *V. negundo* extracts in preventing growth of *S. litura* when applied at ten per cent concentration. The nonazadirachtin limonoids from neem, nimbocinol and azadiradione reduced the efficiency of conversion of ingested food (ECI) in feeding experiments, indicating toxic rather than antifeedant effects (Koul *et al*., 2004a). Similar results were also observed in *H. virescens* treated with fraxinellaone. Treated insects showed significantly reduced activities of ά amylase and non specific proteases there by indicating post ingestive toxicity (Liu *et al*., 2009).

Changes in ECI could be influenced by the digestibility of food, its nutritional status and the level of nutrient intake.

Fig. 13

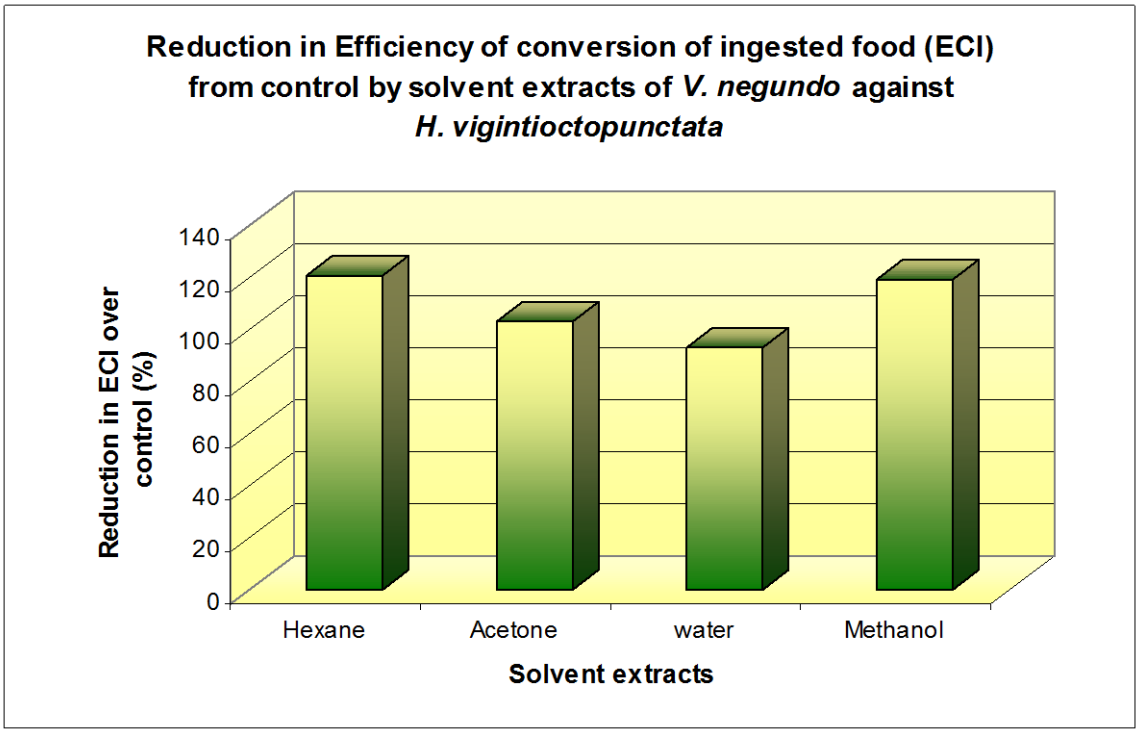
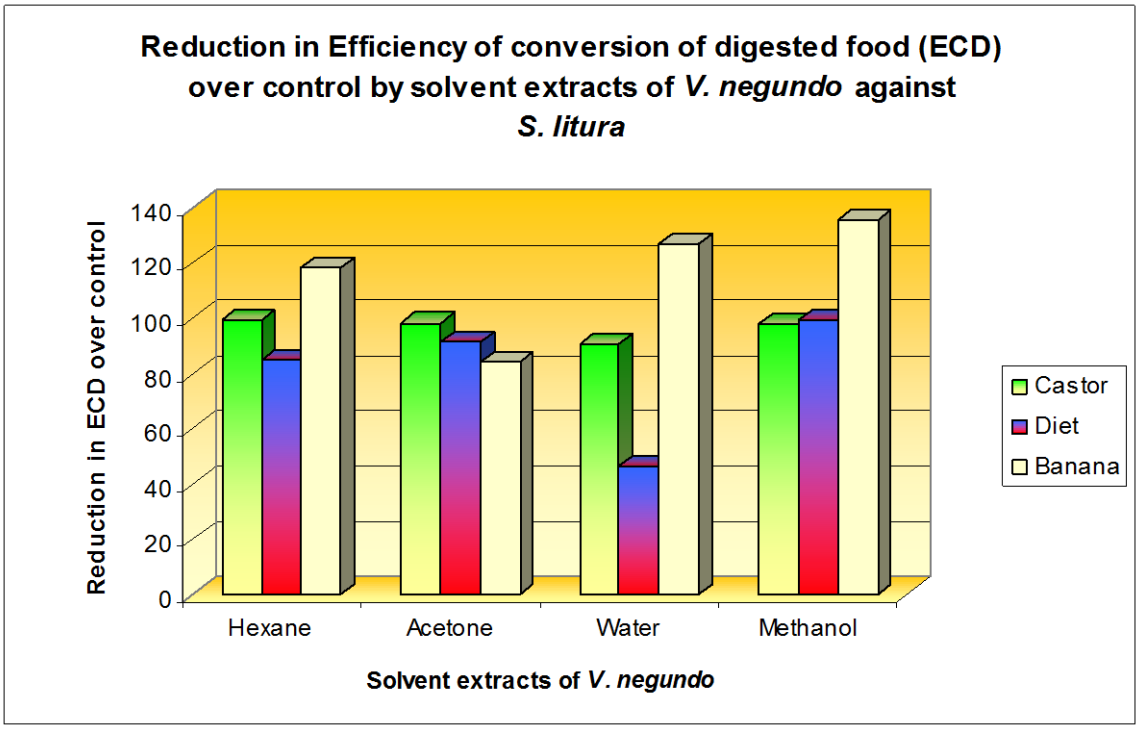

Reduction in Efficiency of conversion of ingested food (ECI) from control by solvent extracts of V. negundo against H. vigintioctopunctata
Reduction in ECI over control (%)
140
120
100
80
60
40
20
0
Hexane
Acetone
water
Methanol
Solvent extracts

Fig. 14


Reduction in Efficiency of conversion of digested food (ECD) over control by solvent extracts of V. negundo against S. litura
Reduction in ECD over control
140
120
100
80
60
40
20
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
Castor
Diet
Banana

5.2.8. Efficiency of conversion of digested food (ECD)

5.2.8.1. ECD as influenced by different solvent extracts of V. negundo against S. litura on castor, semi-synthetic diet and banana

The results of this experiment are given in Table 16 and Fig.14. From this it is clear that the ECD values were also found to be decreased with the increase in concentrations of different solvent extracts indicating the effectiveness of higher concentrations of *V. negundo* extracts in preventing nutrient assimilation of food by *S. litura* larvae. According to Wald-bauer (1968), ECD was decreased as the proportion of digested food metabolized for energy increased. On castor, hexane extract caused lowest ECD (0.482%) with highest reduction of ECD from control (99.26%). Water extract (6%) recorded highest ECD of 4.84 per cent. On semi-synthetic diet methanol extract caused lowest ECD of 0.11 per cent showing its highest potency to reduce the ECD upto 99.66 per cent while water extract caused highest ECD of 15.99 per cent. On banana all extracts except acetone caused negative values of ECD with highest reduction of 135.64 per cent by methanol extract followed by water extract (126.95%) (Table 16 and Fig 14.).

It is thus clear that among the three hosts, ECD values were lowest on banana and highest in semi-synthetic diet.

Efficiency of utilization of food by animals is measured by the ECI and ECD values. In the present study, negative values for both ECI and ECD were observed especially at higher concentrations indicating that utilization of energy for various life activities was challenged by the allelochemicals present in the *V. negundo* extract. Similar finding was also observed by Sahayaraj (1998). From this study it could be inferred that all these four solvent extracts at higher concentrations could be effectively reduce the conversion of digested food. Negative values indicate that they are potential growth inhibitors and interfere in the metabolic conversion processes in insect nutrition.

The increase in ECD was due to less food utilization for energy and more for incorporation into the body matter. The ECI and ECD into body mass was considerably reduced when compared to control. Fagoonee (1984) reported

similar results in *Crocidolmia binotalis* with azadirachtin treated diet. As indicated by Mark and James (1988), the reason for low utilization efficiency in *T. siva* at higher concentrations might be due to the dissipation of energy from the production of body mass to detoxification. Reduction in ECD of rice leaf folder larvae on treatment with *Dysoxylum* triterpenes was reported by Nathan *et al*.(2007). According to them, the consumption and conversion efficiency were highly correlated with the gut enzyme activity of *C. medinalis*. Tetranortriterpenoid contains enzyme inhibiting components, which reduced the conversion rate of food (Broadway and Duffey 1988., Breuer *et al*., 2003 and Huang *et al*., 2004). The percentage reduction in ECI and ECD results from a food conversion deficiency, which reduces growth through a diversion of energy from biomass production into detoxification (Wheeler *et al*., 2001). The gut enzymes, acid phosphatases (ACP), alkaline phosphatases (ALP) and adenosine triphosphatases (ATP ase) were significantly inhibited by the *Dysoxylum* triterpenes (Nathan *et al*., 2007). Treatment with neem limonoids and *M. azedarach* leaf extract caused a drastic decline in the activity of ALP and ACP (Nathan 2006). Koul *et al*., (1995) also found that azadirachtin interferes with growth via digestive impairement by inhibiting the secretion of trypsin like proteases from gut epithelial cells. Similar results were also reported by Timmins and Reynolds (1992) in *Manduca sexta* L. Histological studies in the alimentary canal of *S. litura* larvae treated with botanicals resulted in a heavy damage of epithelial cells and circular muscles in annona seed extract treated larvae than the neem treated larvae (Vijayaraghavan *et al*., 2003). Protein content of *S. litura* was reduced considerably on treatment with annona seed extracts (Vijayaraghavan *et al*., 2004).

ECD also decreases as the proportion of digested food metabolized for energy increases. Decrease in ECI and ECD values indicates that *V. negundo* extracts excert chronic toxicity to insects. Secondary metabolite in *V. negundo* extracts interfered with the digestion and / or absorption of ingested food and with the conversion of absorbed food to biomass.

5.2.8.2. ECD as influenced by different solvent extracts of V. negundo against H. vigintioctopunctata

ECD of *H. vigintioctopunctata* was found to be decreased with increase in *V. negundo* extract concentrations. Hexane extract (6%) showed lowest (-7.31%) ECD with the highest reduction of 120.36 per cent in *H. vigintioctopunctata* and highest ECD was recorded in water extract (6%) with a reduction of 29.89 per cent when compared to control. All the extracts except water, recorded more than 100 per cent reduction in ECD when compared to control (Table17 and Fig.15). All these extracts could be used as potent growth inhibitors of *H. vigintioctopunctata*. According to Kumar and Ahmed (2006), ECD was affected by the amount of energy devoted for maintenance of physiological functions of the animal. Results of the current investigation is in concurrence with the findings of the experiments conducted by Kumar and Ahmed (2006). Decrease in ECD could be explained by a higher metabolic cost of processing of food which contains allelochemicals. Processing costs are associated with induction mechanisms at the level of digestion and detoxification.

5.2.9. Relative consumption rate (RCR)

5.2.9.1. RCR as influenced by different solvent extracts of V. negundo against S. litura on castor, semi-synthetic diet and banana

Results are presented in Table18 and Fig.16.

On castor, solvent extracts of *V. negundo* at higher concentrations (4 and 6%) resulted in lower RCR values when compared to control. Among the four types of *V. negundo* extracts, acetone extract caused maximum reduction in consumption (65.07%) followed by water extract (57.87%) (Fig.16).

When *S. litura* larvae were reared on semi-synthetic diet, all the solvent extracts except acetone at four per cent recorded higher consumption rates compared to control. Maximum increase in RCR was recorded by hexane extract (63.38%) and minimum by acetone extract (2.21%). Similar results were observed

Fig. 15

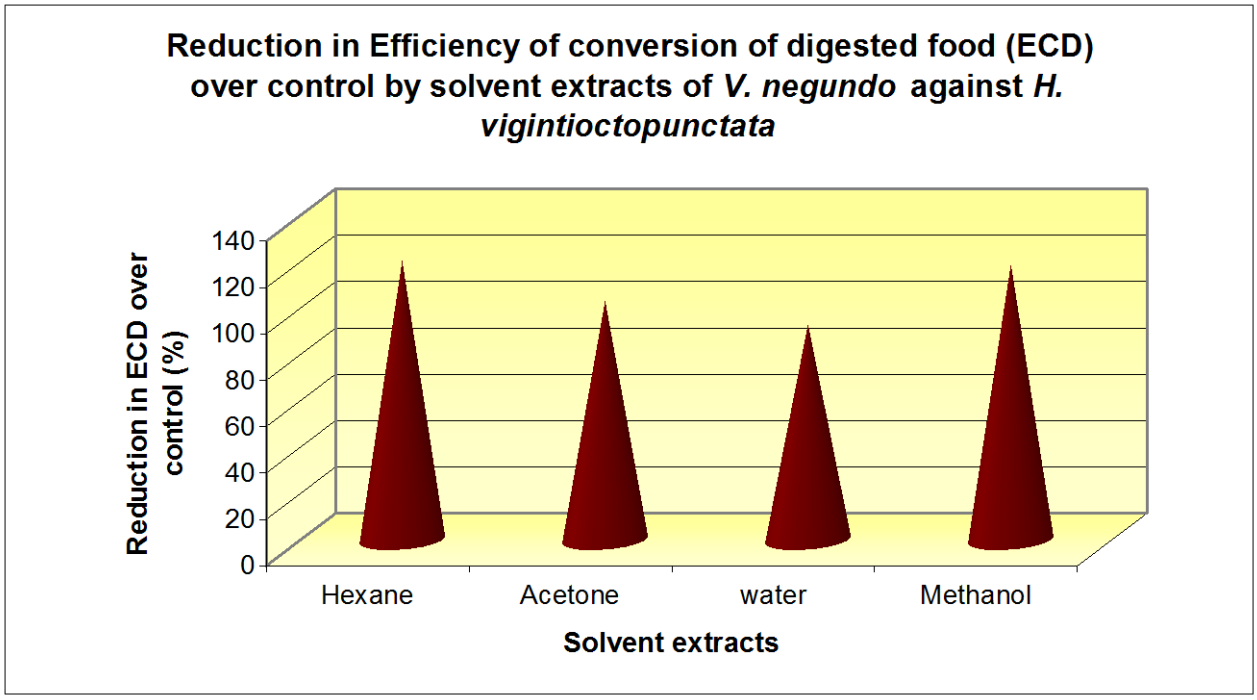
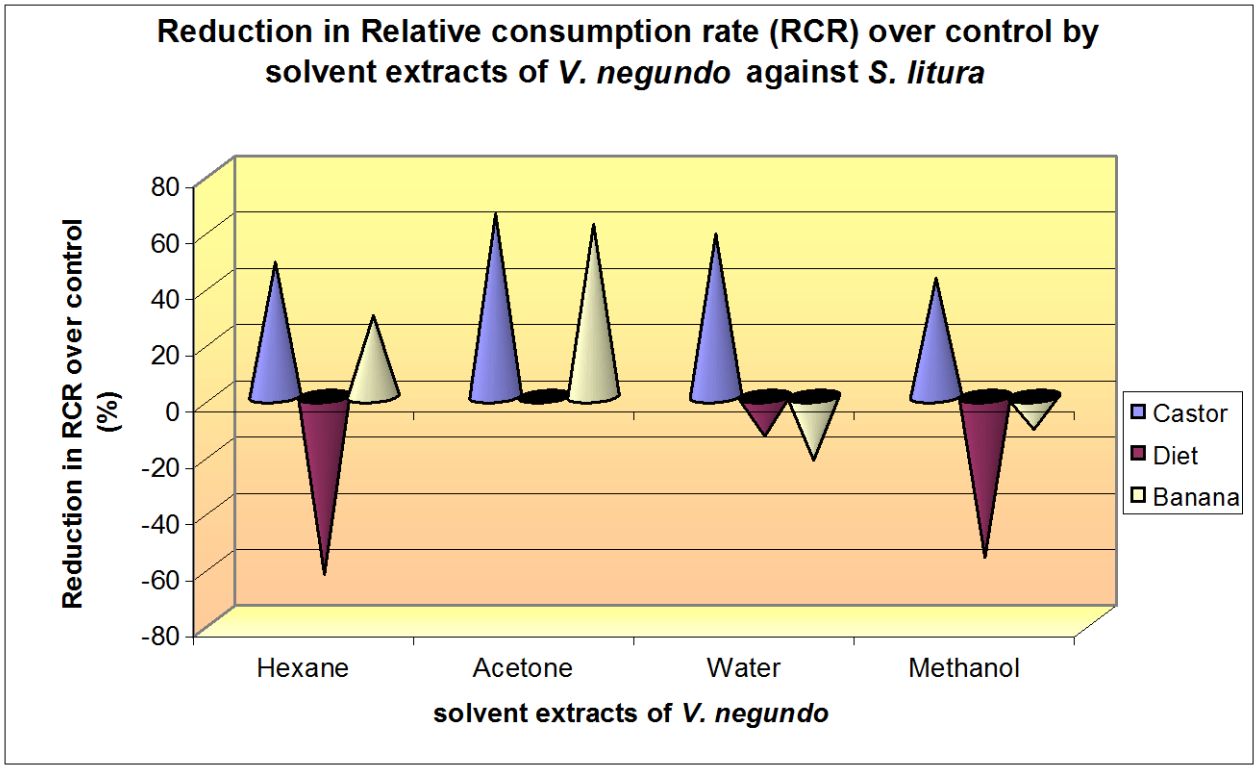

Reduction in Efficiency of conversion of digested food (ECD) over control by solvent extracts of V. negundo against H. vigintioctopunctata
Reduction in ECD over control (%)
140
120
100
80
60
40
20
0
Hexane
Acetone
water
Methanol
Solvent extracts

Fig.16


Reduction in Relative consumption rate (RCR) over control by solvent extracts of V. negundo against S. litura
Reduction in RCR over control (%)
80
60
40
20
0
-20
-40
-60
-80
Hexane
Acetone
Water
Methanol
solvent extracts of V. negundo
Castor
Diet
Banana

by Mohamed *et al*. (2003) where in azadirachtin (1250, 625, 312.50, 100 ppm) treated larvae recorded higher RCR (41.5, 44.40, 48.20, and 31.90% increase) when compared to control.

Aqueous extract increased RCR when *S. litura* was fed with diet and banana where as RCR was reduced by 57.87% when aqueous extract treated castor was fed to *S. litura*. In case of both diet and banana reared *S. litura* larvae, aqueous extract increased RCR, whereas RCR was reduced to 57.87 per cent when aqueous extract treated castor was fed to *S. litura*. When *S. litura* larvae were reared on banana, maximum per cent reduction in RCR was obtained by acetone extract at six per cent with 61.33 per cent reduction in RCR when compared to control. Reduced consumption (79.4% reduction from control) of the rice leaf folder larvae on treatment with *Dysoxylum* triterpenes were reported by Nathan *et al*., 2007.

A comparison of the RCR of *S. litura* reared on three hosts treated with *V. negundo* indicated a lowest value in banana and highest in semi-synthetic diet.

5.2.9.2. RCR as influenced by different solvent extracts of V. negundo against H. vigintioctopunctata

At six per cent concentration, all the solvent extracts reduced RCR when compared to control. Maximum reduction in RCR was recorded by acetone extract (50.05%) followed by hexane extract (42.17%). Aqueous extract resulted in lowest reduction in RCR (16.16%) over control (Fig.17). The efficacy of acetone extract of *V. negundo* in inhibiting the consumption of *H. vigintioctopunctata* is thus indicated in the present finding.

5.2.10. Larval growth

5.2.10.1. Larval growth as influenced by different solvent extracts of V. negundo against *S. litura on castor, semi-synthetic diet and banana*

Larval growth was examined in different treatments on fresh weight basis. Results are presented in Table 20 and Fig.18.

Larval growth showed a reducing trend with increase in concentration.

On castor, Acetone, hexane and methanol extracts at six per cent recorded maximum growth reduction when compared to control (108.11, 97.22 and 97.44 % respectively) (Fig.18). Lowest larval growth was recorded by the solvent extracts of acetone at six per cent concentration with a mean value of -0.003. Negative value indicates that instead of weight gain, loss of weight had happened. It was followed by hexane and methanol extracts at same level with a mean production value of 0.001 each (Table 20). Experiments conducted by Koul *et al*.,(2004b) reported that nimbocinol exhibited growth inhibitory activity in artificial diet bioassays with 82.4 and 92.2 mg kg^{-1} concentrations inhibiting growth by 50 per cent when tested against *H. armigera* and *S. litura*. From this it is clear that the treated larvae were unable to utilize the consumed food for its growth. Most of the energy was utilized for the degradation of the toxic metabolite present in the extract.

V. negundo extracts treated diet when fed to *S. litura* larvae resulted in reduction in larval growths over control at six per cent concentrations. Larval growth was completely stopped with extracts of methanol at six per cent concentration. Aqueous extracts recorded very low larval growth at higher levels (4 per cent) with growth inhibition of 51.22 per cent when compared to control.

When the larvae were reared on banana all the solvent extracts (6%) except methanol recorded more than 80 per cent growth reduction and highest growth inhibition was exhibited by acetone extract (92.86%) (Fig.18).

Among the four extracts of *V. negundo*, acetone extract reduced growth of *S. litura* larvae upto the highest extend (69.86 to 108.11%). Aqueous extract caused lowest reduction (21.95 to 90%) in larval growth. Larvae reared on *V. negundo* treated castor suffered highest weight reduction ranged from 74.29 to 108.11 per cent. *V. negundo* treated diet resulted in a lower reduction of larval growth (12.5 to 100%).

Growth of the larvae differed significantly with different hosts. Highest larval growth was observed in semi-synthetic diet followed by castor and banana. Effect of different solvent extracts of *V. negundo* differed significantly with

Fig. 17

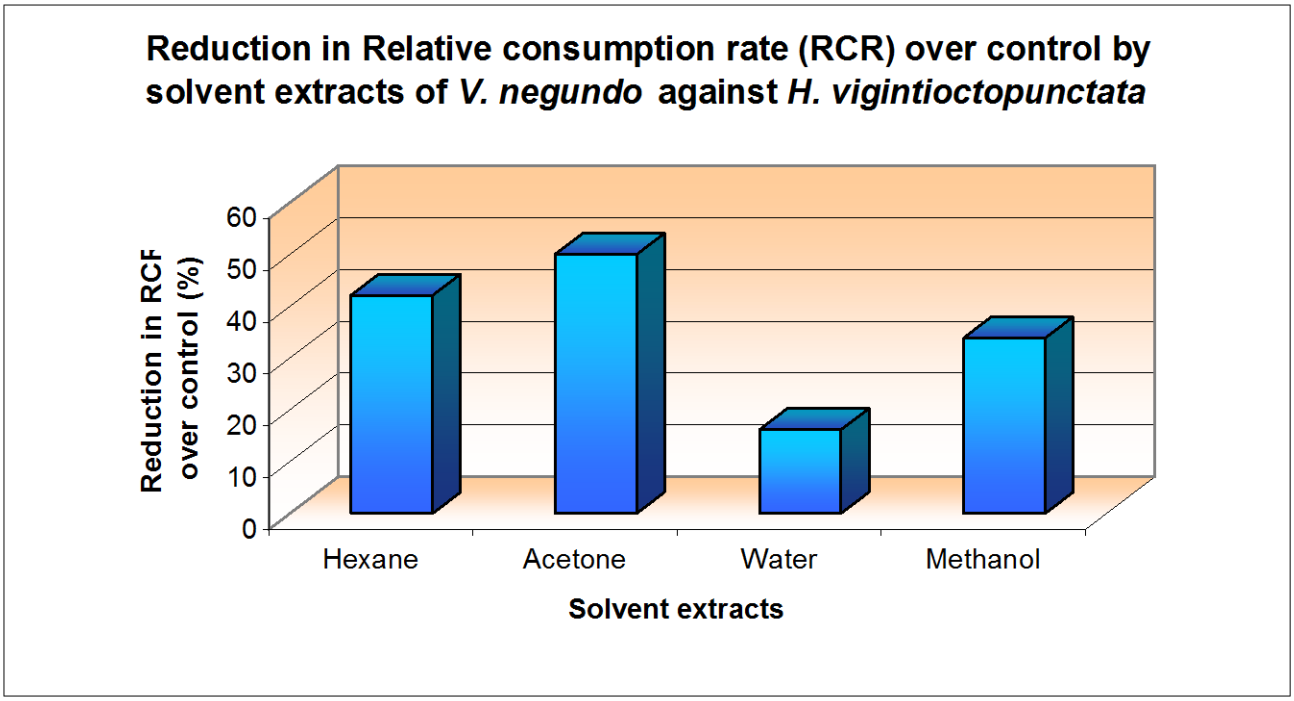
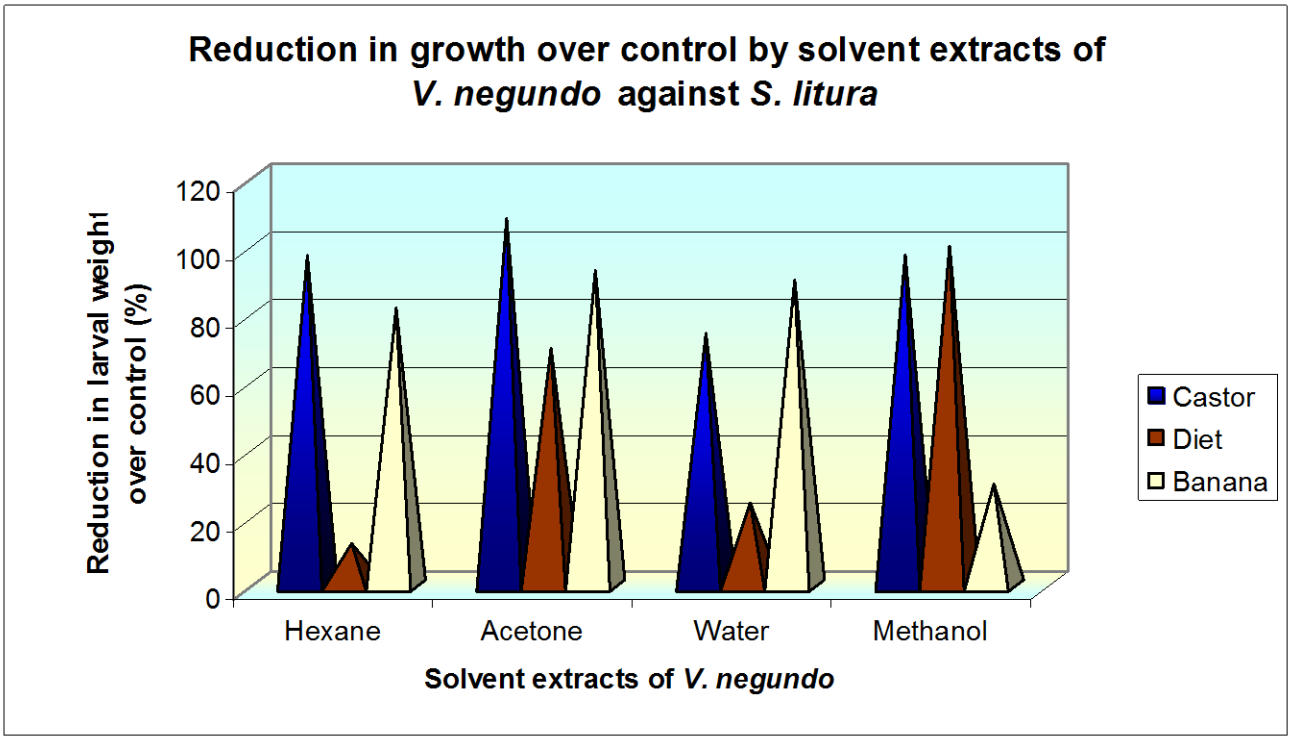

Reduction in Relative consumption rate (RCR) over control by solvent extracts of V. negundo against H. vigintioctopunctata
Reduction in RCF over control (%)
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts

Fig. 18


Reduction in growth over control by solvent extracts of V. negundo against S. litura
Reduction in larval weight over control (%)
120
100
80
60
40
20
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
Castor
Diet
Banana

different hosts. In case of castor, acetone at six per cent level acted as a potent growth inhibitor, while in case of semi-synthetic diet and in banana, methanol extracts at six per cent level resulted in significant growth inhibition. In case of banana aqueous extracts at six per cent level also acted as a potent growth inhibitor. The growth and consumption of lepidopterans, *S. litura* and *T. ni* were negatively affected by the presence of furanoquinoline alkaloids in the diet (Sacket *et al*., 2007)

5.2.10.2. Larval growth as influenced by different solvent extracts of V. negundo against H. vigintioctopunctata

There was no significant difference in larval growths between different solvents in the control treatment indicating that the solvents alone had no inhibitory effect on larval growth of *H. vigintioctopunctata***.**

Growth of grubs showed a declining trend towards increase in concentrations. Lowest growth was recorded in the hexane extracts at six per cent concentration with a mean value of -0.016. It was followed by methanol extracts at six per cent concentration with mean production value of -0.010. Negative value indicates that instead of weight gain, loss of weight had happened. Maximum growth reduction of 161.54 per cent and 133.33 per cent was recorded by hexane and methanol extracts (6%) (Table 21 and Fig.19). Treated larvae were unable to utilise the ingested food for its body mass. Acetone extracts also at six per cent level recorded complete inhibition of growth and was on par with the same solvent at four per cent level.

Solvent extracts of hexane, methanol and acetone at six per cent level acted as potent growth inhibitors against *H. vigintioctopunctata*. All solvent extracts at higher levels effectively prevented growth. Even though the consumption was higher, it was not reflected in the growth indicating post ingestive toxicity interfering with the metabolic activities. According to Kumar and Ahmed (2006), increased rate of consumption was due to increase in metabolic activities. They had stated that consumption index showed a statistically non-significant negative correlation with ECI and also with ECD. Significant positive correlation was

found between RGR and ECI as well as ECI and ECD. It indicated that increased intake of food enhanced the ejection and metabolism.

5.2.11. Relative Growth Rate (RGR)

RGR indicates the rate at which the digested food is available to the insect and ultimately the rate of increase in weight of insect body in a unit time period. (Kumar and Ahmed, 2006).

5.2.11.1. RGR as influenced by different solvent extracts of V. negundo against S. litura on castor, artificial diet and banana

RGR in different treatments were calculated and results are presented in Table 22 and Fig.20.

On castor, all treatments at all concentrations recorded lower RGR values when compared to control. Solvent extracts of acetone and methanol at six per cent level completely inhibited growth of larvae and resulted in loss of weight (-0.079 and -0.003gday^{-1}) instead of weight gain indicating the efficacy of these solvent extracts as potential growth inhibitors (Table 22). Solvent extracts with hexane also recorded lower RGR value of 0.026 at six per cent concentration. Highest growth reduction (110.59%) was observed by acetone extract (6%) followed by methanol extract (100.33%). Hexane extract also recorded more than 95 per cent growth inhibition at six per cent (Fig.20).

When the larvae were reared on semi-synthetic diet, all the solvent extracts recorded relatively lower RGR values at higher concentrations. Lowest RGR and highest growth reduction (99.53 %) was recorded by methanol extract at six per cent level while water extract resulted in lowest reduction (26.45 %) in RGR. All the solvent extracts except water at six per cent level recorded more than 50 per cent inhibition of growth when compared to control.

When the experiment was conducted on banana, lowest RGR value and maximum growth reduction (133.94%) was obtained by methanol extracts at six per cent level followed by water extracts (129.47%). Hexane and acetone extracts at six per cent also recorded more than 80 per cent growth inhibition. This

Fig. 19

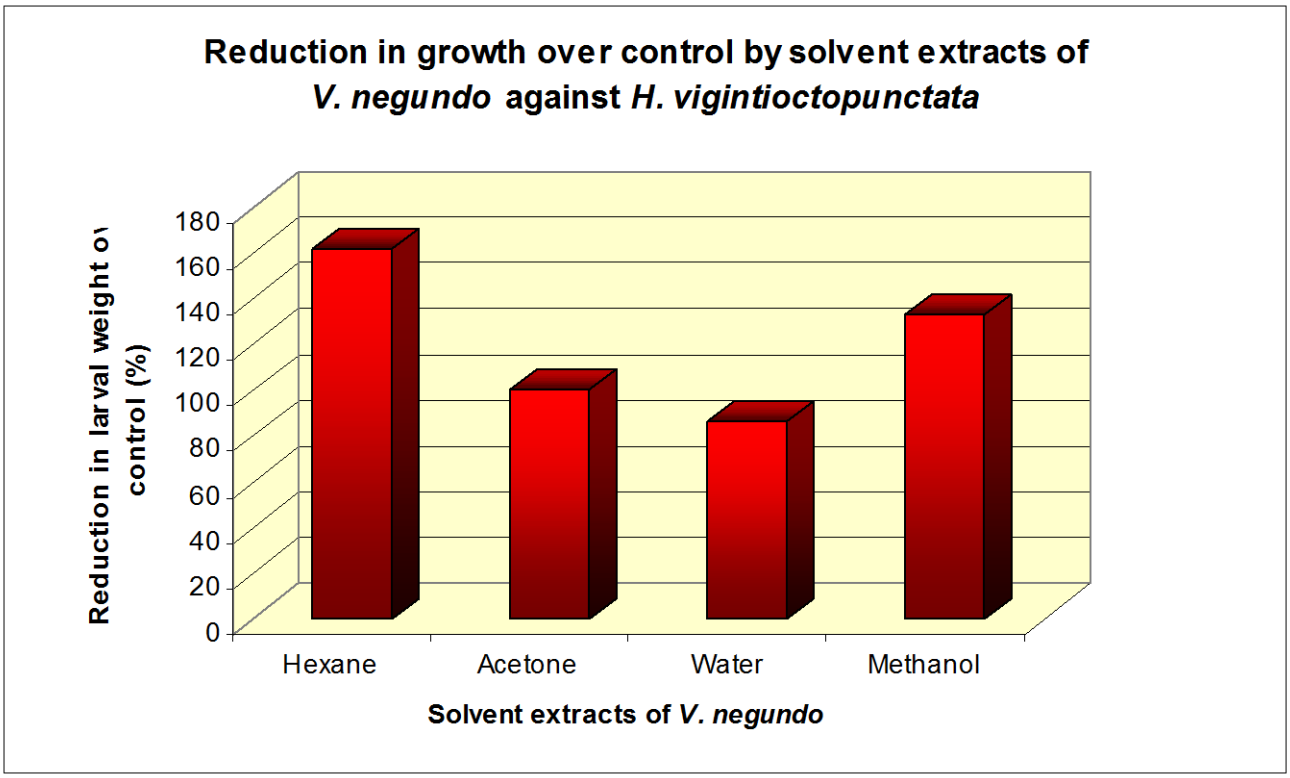
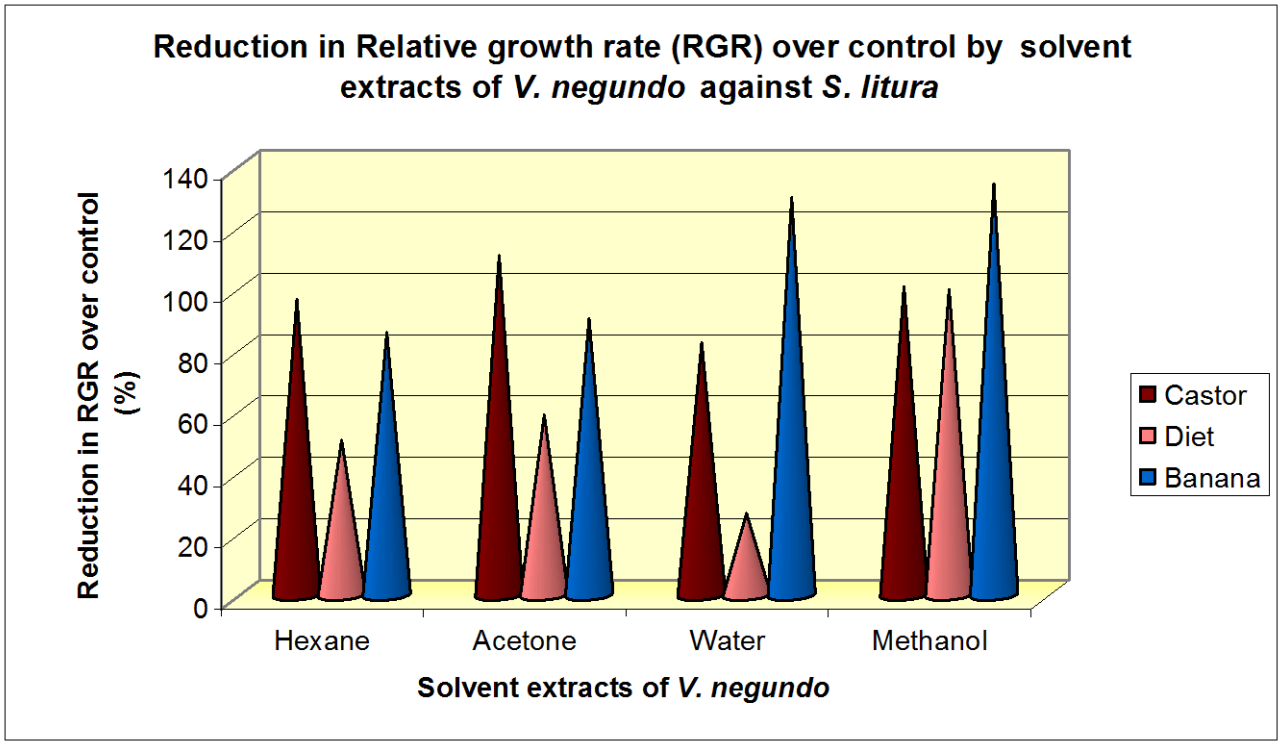

Reduction in growth over control by solvent extracts of V. negundo against H. vigintioctopunctata
Reduction in larval weight over control (%)
180
160
140
120
100
80
60
40
20
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo

Fig. 20


Reduction in Relative growth rate (RGR) over control by solvent extracts of V. negundo against S. litura
Reduction in RGR over control (%)
140
120
100
80
60
40
20
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo
Castor
Diet
Banana

is in accordance with the findings of Koul *et al*. (2004b) wherein nimbocinol a neem limonoid exhibited growth inhibitory activity against *S. litura* and *H. armigera* by reducing growth to 50 per cent compared to control.

In all the hosts, methanol extracts acted as potent growth inhibitors at higher concentrations (4 and 6%). Acetone extract of *V. negundo* also proved as efficient growth inhibitors against *S. litura* larvae reared in both castor and banana. Aqueous extract at higher concentrations (4 and 6%) completely inhibited larval growth and proved its potency as an efficient growth inhibitor against *S. litura* larvae reared on banana. However, its effect was not much pronounced in larvae reared on semi-synthetic diet.

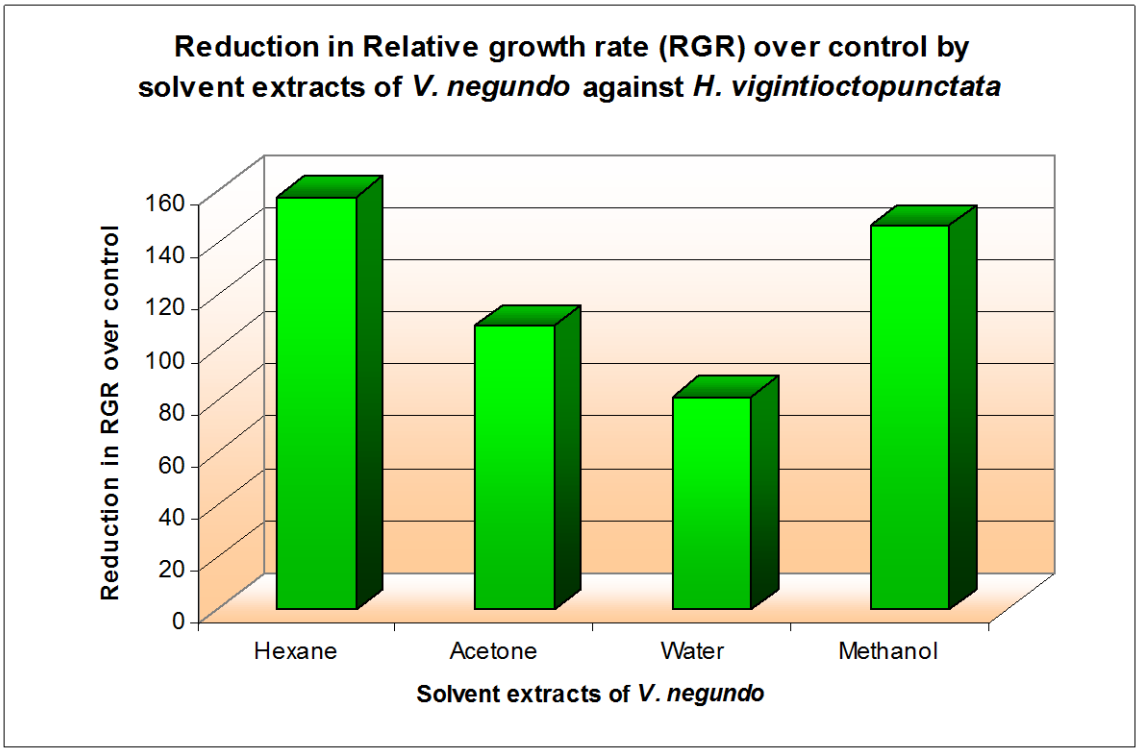
Reduction in growth of larvae was not entirely due to starvation, but partly due to the toxic effects of the ingested *V. negundo* extracts. This in confirmation with the findings of Sahayaraj (1998) where he reported that maximum mean reduction per cent (73.69) in body weight was found in insects treated with *V. negundo* extract. He has indicated that the reduction in growth might be due to less or slow feeding of the foliage and diversion of energy from production of biomass to detoxification of plant extracts. Similar results were also observed by Koul *et al*., (2005) where growth inhibition of 95 per cent was achieved on treatment with 2.36 ppm of aglaoroxin A against *S. litura* larvae. The treatment of *Dysoxylum* triterpenes into the rice leaves significantly reduced larval growth of rice leaf folder when compared to control. RGR was decreased upto 54.2 per cent at 12 ppm concentration (Nathan *et al*., 2007).

Results of the present study indicates that all the solvent extracts except methanol were having some influence on the hosts on which the larvae were reared.

5.2.11.2. RGR as influenced by different solvent exracts of V. negundo against H. vigintioctopunctata

At higher concentrations all the solvents recorded lower RGR values as evidenced from the Table 23 and Fig.21. The solvent extracts with hexane and methanol recorded lowest RGR values of -0.266 and -0.231 at six per cent level.

Fig. 21


Reduction in Relative growth rate (RGR) over control by solvent extracts of V. negundo against H. vigintioctopunctata
Reduction in RGR over control
160
140
120
100
80
60
40
20
0
Hexane
Acetone
Water
Methanol
Solvent extracts of V. negundo

At the highest concentration (6%) all the solvent extracts recorded more than 100 per cent growth inhibition when compared to control and maximum inhibition (157.58 %) was recorded by hexane extract.

Present investigation considerably substantiates the hypothesis that the natural plant products like *V. negundo* extracts may have chronic effects on rate of growth, ingestion and utilization of food by herbivores. Nutritional index experiments showed that methanol extracts had adverse effect on all the nutritional indices.

Results of this experiment clearly indicate the adverse effect of *V. negundo* inducing physiological changes that may reduce the nutritional indices, survival and growth of *S. litura* and *H. vigintioctopunctata*.

5.2.11.3 Correlation of RGR of S. litura in different treatments with RCR, ECI and ECD on different hosts

Correlation studies were conducted to determine the exact factor which contributes to the growth in insects. Results are presented in Table 24 and Figs.22 to 27.

In case of castor reared larvae, RCR is not showing any definite relationship with RGR. There is no direct relationship with RCR and RGR. It is evident from the figure 22. But, in case of the nutritional indices, ECI and ECD, RGR shows a definite trend showing direct relationship (Fig.23). When the ECI and ECD were lower, RGR also showed a reduction and when these indices were increased, RGR also increased. Significant positive correlation was found between ECI and ECD with RGR (Table 22). Similar trends were observed when the larvae were reared on semi-synthetic diet and banana (Figs.24 to 27). From this it is concluded that even when the consumption is high it need not be reflected on the growth. RGR is positively influenced by increased assimilation and conversion into body substance of the larvae. Results of the present study were in consonance with the findings of Kumar and Ahmed, 2006. According to them, consumption index showed a statistically non significant negative correlation with ECI and ECD.

Fig. 24

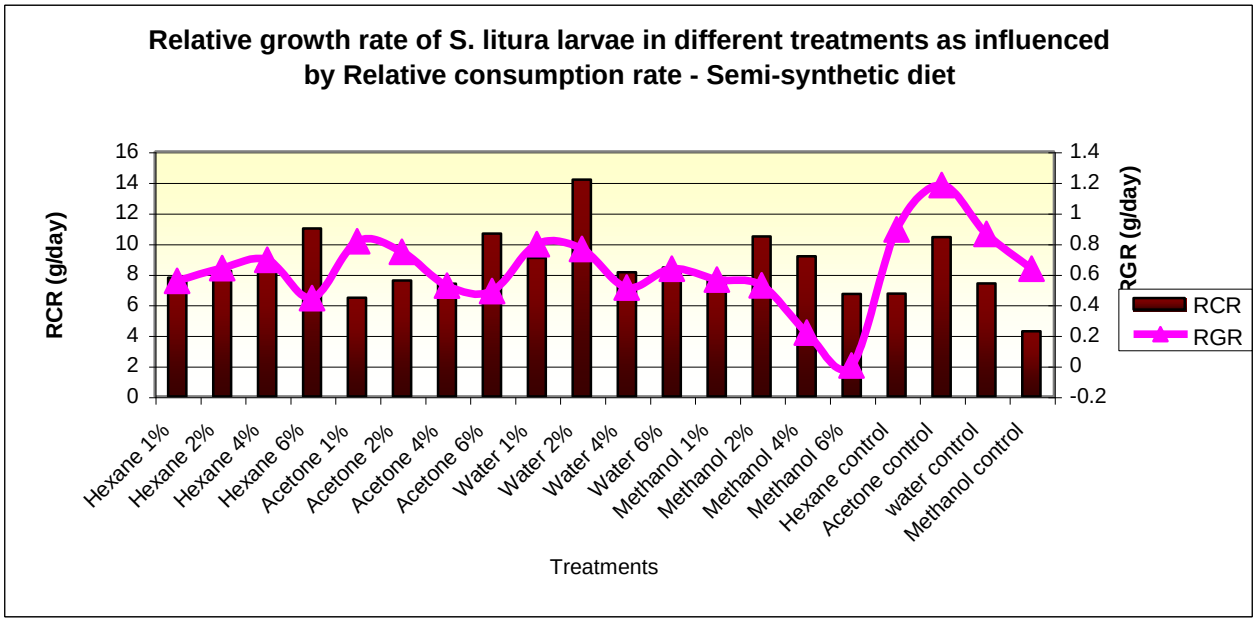

Relative growth rate of S. litura larvae in different treatments as influenced by Relative consumption rate - Semi-synthetic diet
RCR (g/day)
RGR (g/day)
Treatments
RCR
RGR
Hexane 1%
Hexane 2%
Hexane 4%
Hexane 6%
Acetone 1%
Acetone 2%
Acetone 4%
Acetone 6%
Water 1%
Water 2%
Water 4%
Water 6%
Methanol 1%
Methanol 2%
Methanol 4%
Methanol 6%
Hexane control
Acetone control
water control
Methanol control

Fig. 25

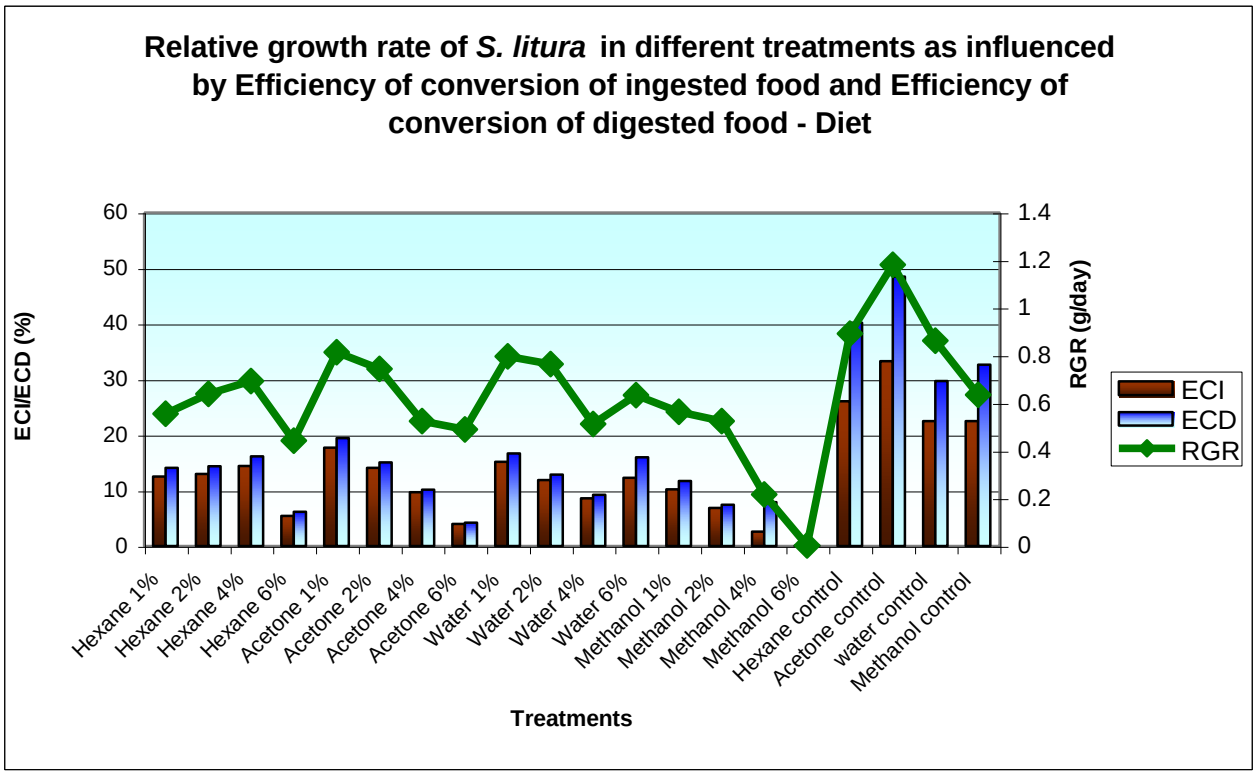

Relative growth rate of S. litura in different treatments as influenced by Efficiency of conversion of ingested food and Efficiency of conversion of digested food - Diet
ECI/ECD (%)
RGR (g/day)
Treatments
ECI
ECD
RGR
Hexane 1%
Hexane 2%
Hexane 4%
Hexane 6%
Acetone 1%
Acetone 2%
Acetone 4%
Acetone 6%
Water 1%
Water 2%
Water 4%
Water 6%
Methanol 1%
Methanol 2%
Methanol 4%
Methanol 6%
Hexane control
Acetone control
water control
Methanol control

Fig. 26

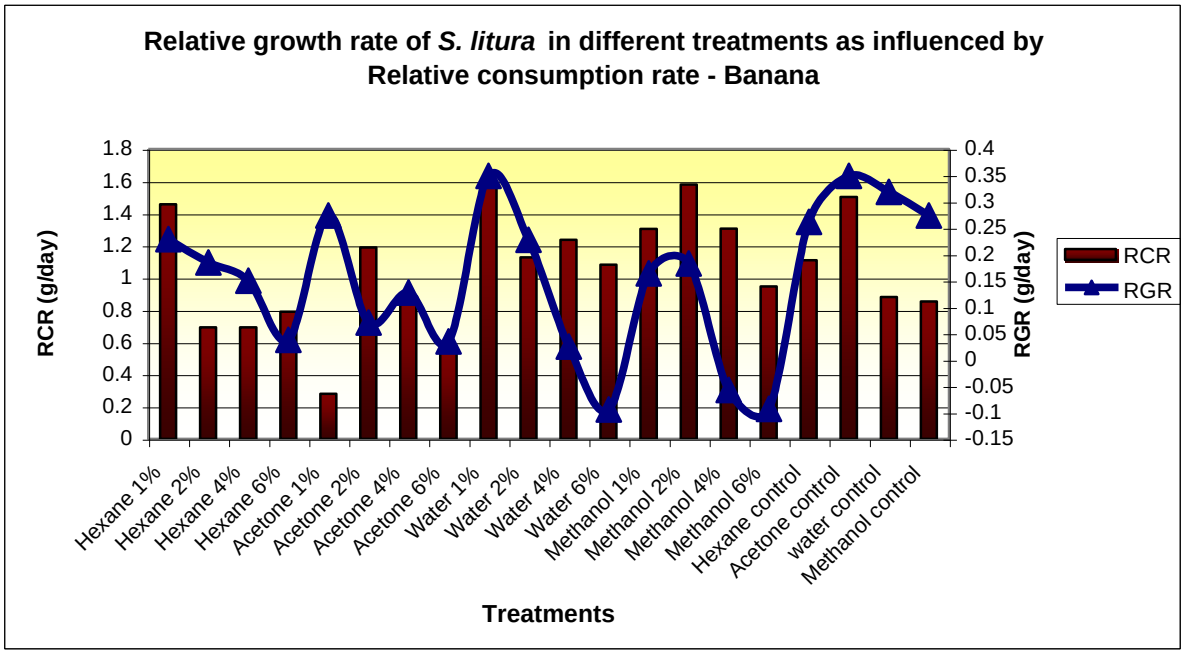

Relative growth rate of S. litura in different treatments as influenced by Relative consumption rate - Banana
RCR (g/day)
RGR (g/day)
Treatments
RCR
RGR
Hexane 1%
Hexane 2%
Hexane 4%
Hexane 6%
Acetone 1%
Acetone 2%
Acetone 4%
Acetone 6%
Water 1%
Water 2%
Water 4%
Water 6%
Methanol 1%
Methanol 2%
Methanol 4%
Methanol 6%
Hexane control
Acetone control
water control
Methanol control

Fig. 27

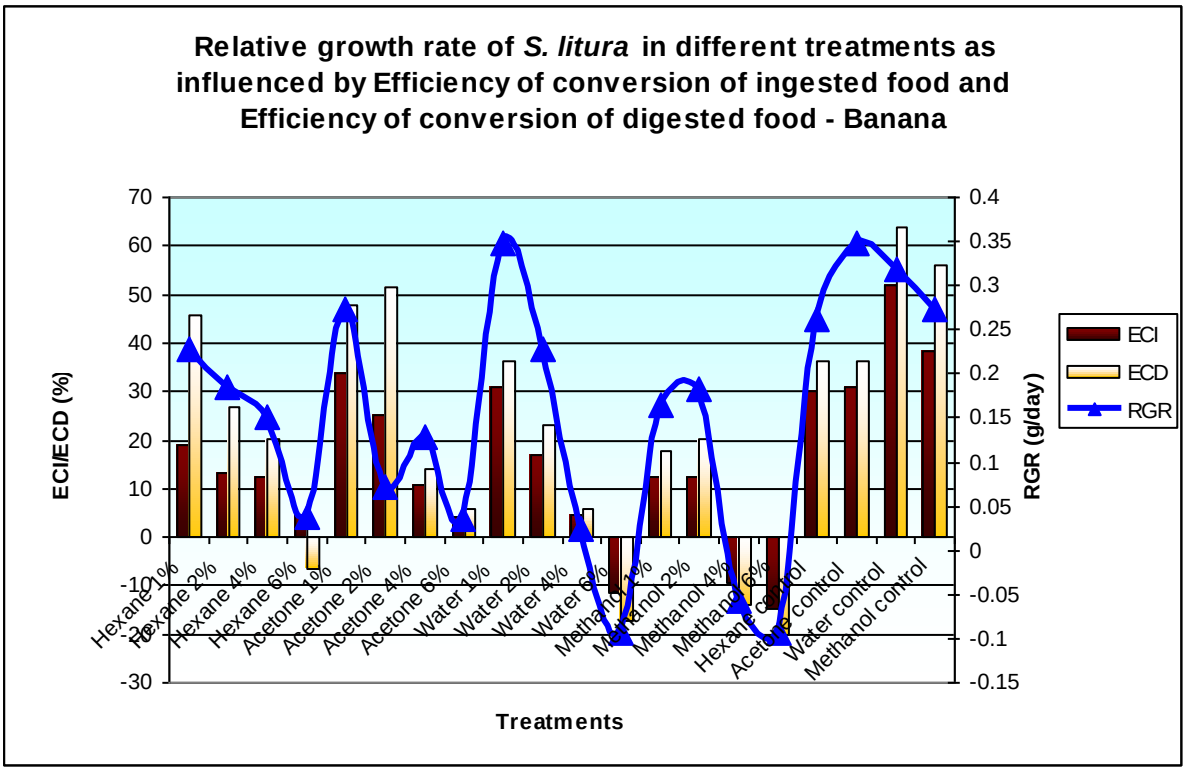

Relative growth rate of S. litura in different treatments as influenced by Efficiency of conversion of ingested food and Efficiency of conversion of digested food - Banana
ECI/ECD (%)
RGR (g/day)
Treatments
ECI
ECD
RGR
Hexane 1%
Hexane 2%
Hexane 4%
Hexane 6%
Acetone 1%
Acetone 2%
Acetone 4%
Acetone 6%
Water 1%
Water 2%
Water 4%
Water 6%
Methanol 1%
Methanol 2%
Methanol 4%
Methanol 6%
Hexane control
Acetone control
Water control
Methanol control

Fig. 28

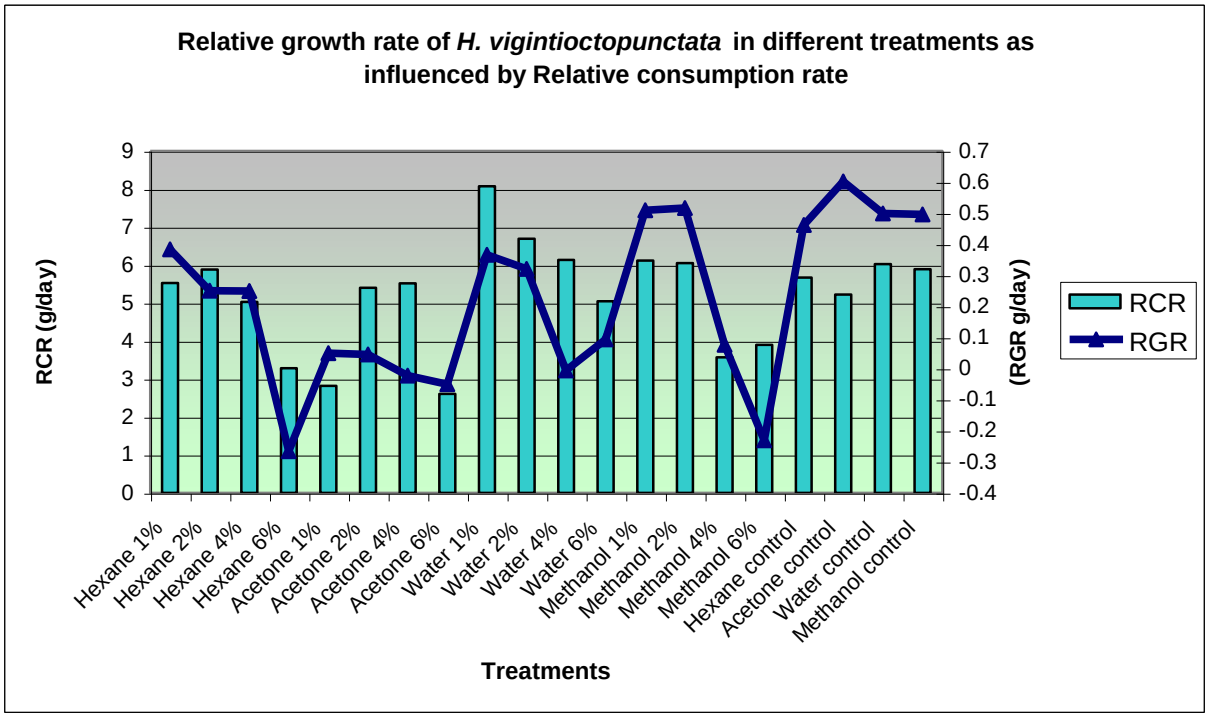
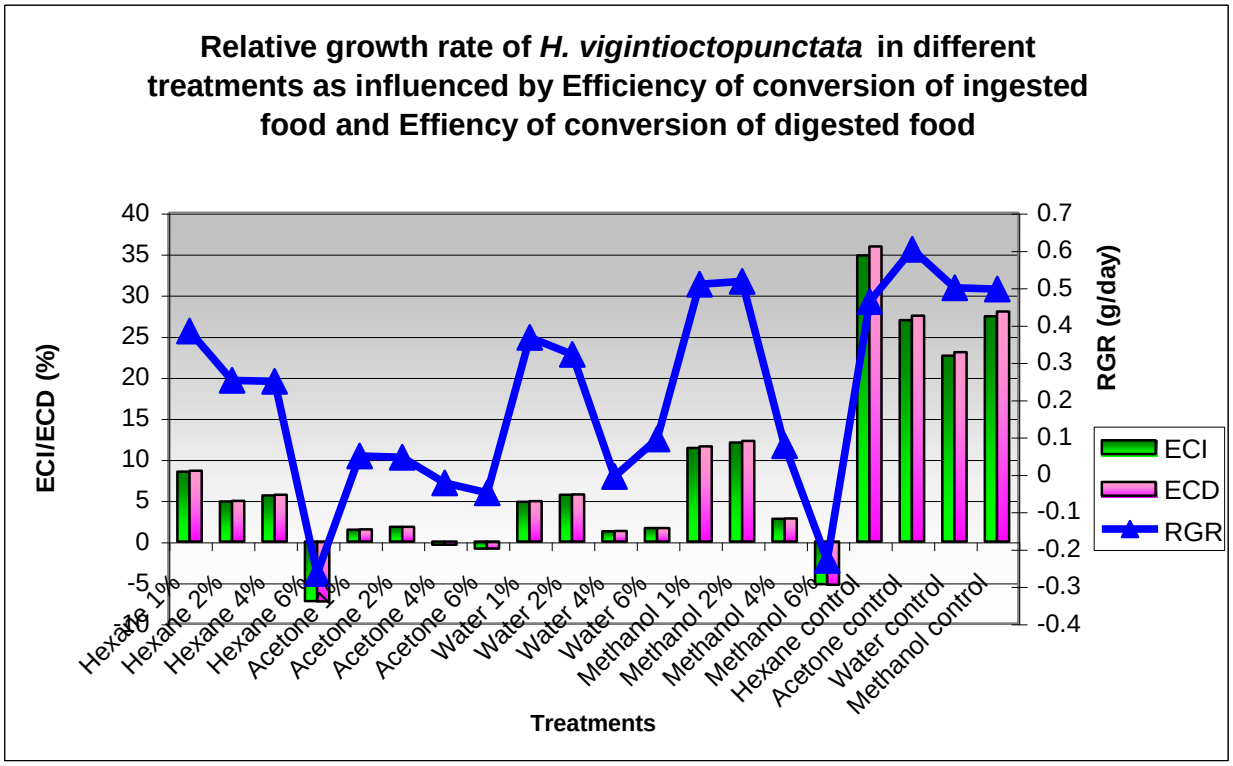

Relative growth rate of H. vigintioctopunctata in different treatments as influenced by Relative consumption rate
RCR (g/day)
(RGR g/day)
RCR
RGR
Hexane 1%
Hexane 2%
Hexane 4%
Hexane 6%
Acetone 1%
Acetone 2%
Acetone 4%
Acetone 6%
Water 1%
Water 2%
Water 4%
Water 6%
Methanol 1%
Methanol 2%
Methanol 4%
Methanol 6%
Hexane control
Acetone control
Water control
Methanol control
Treatments

Fig. 29


Relative growth rate of H. vigintioctopunctata in different treatments as influenced by Efficiency of conversion of ingested food and Effiency of conversion of digested food
ECI/ECD (%)
RGR (g/day)
ECI
ECD
RGR
Hexane 1%
Hexane 2%
Hexane 4%
Hexane 6%
Acetone 1%
Acetone 2%
Acetone 4%
Acetone 6%
Water 1%
Water 2%
Water 4%
Water 6%
Methanol 1%
Methanol 2%
Methanol 4%
Methanol 6%
Hexane control
Acetone control
Water control
Methanol control
Treatments

Significant positive correlation was found between RGR and ECI and also RGR with ECD.

5.2.11.4 Correlation of RGR of H. vigintioctopunctata in different treatments with RCR, ECI and ECD

In case of *H. vigintioctopunctata*, RCR showed a positive correlation with RGR but it was not statistically significant. Significant positive correlation was obtained for the nutritional indices ECI and ECD with RGR (Table 25 and Figs. 28 and 29).

5.2.12. Insect growth regulatory (IGR) effect

Insect growth and development are influenced by neurohormones, ecdysone and juvenile hormone. The titre of these hormones determine growth and metamorphosis in insects. Any alterations in the level of these hormones will disrupt the normal growth and thereby leads to morphogenic malformations like larval, pupal, adult malformations, larval pupal intermediaries etc.

An experiment was laid out as per 3.4.2 to determine the morphogenic malformations if any produced by the solvent extracts of *V. negundo*. Results of this experiment are presented in Tables 26 and 27 and Figs.30 to 35.

5.2.12.1. Morphogenic malformations as influenced by different solvent extracts of V. negundo against S. litura on castor, banana and semi-synthetic diet

A perusal of data on morphogenic malformations revealed that total pupation and adult emergence decreased with increase in concentrations of *V. negundo* extracts while pupal and adult malformations showed an increasing trend with increase in concentrations (Table 26).

At the highest concentration (6%), in castor, lowest pupation was recorded by hexane extract (33.33%) and pupal malformations were highest in methanol extract (26.67%). It was followed by hexane extract (23.33%). Adult emergence was lowest in hexane extract (23.33%) and was followed by methanol extract (33.33%). Highest malformed adults were observed in methanol extract (30%) (Figs.30 & 31). Among all the solvent extracts hexane and methanol

Fig. 30

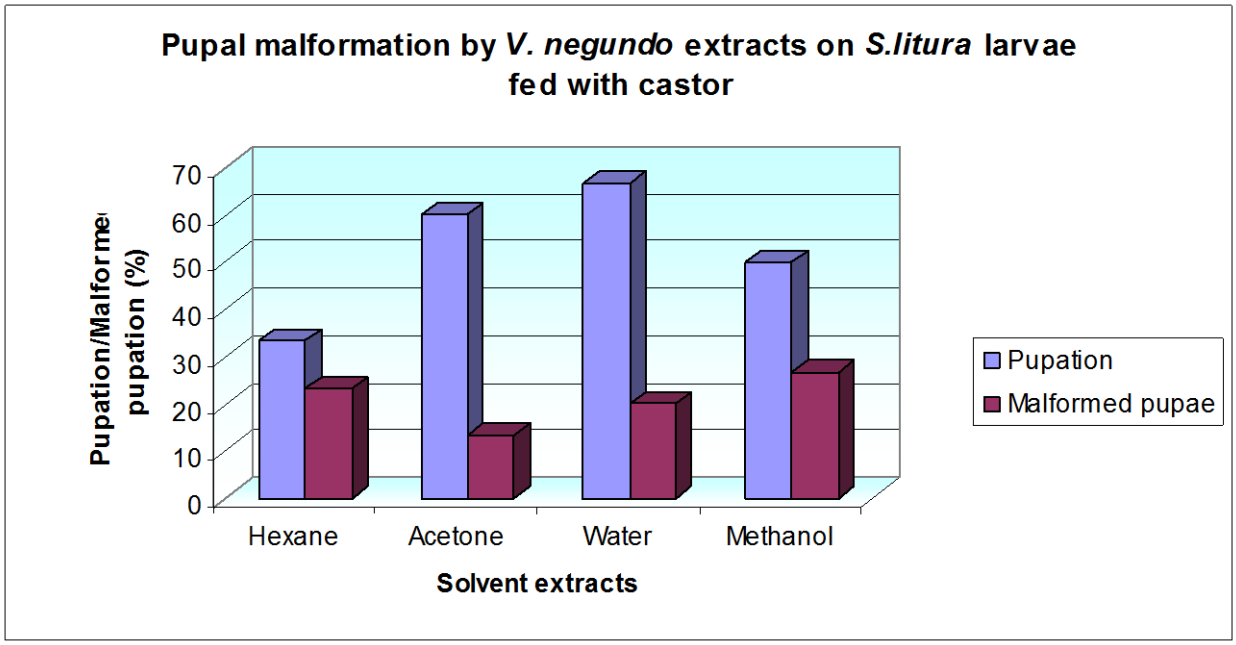
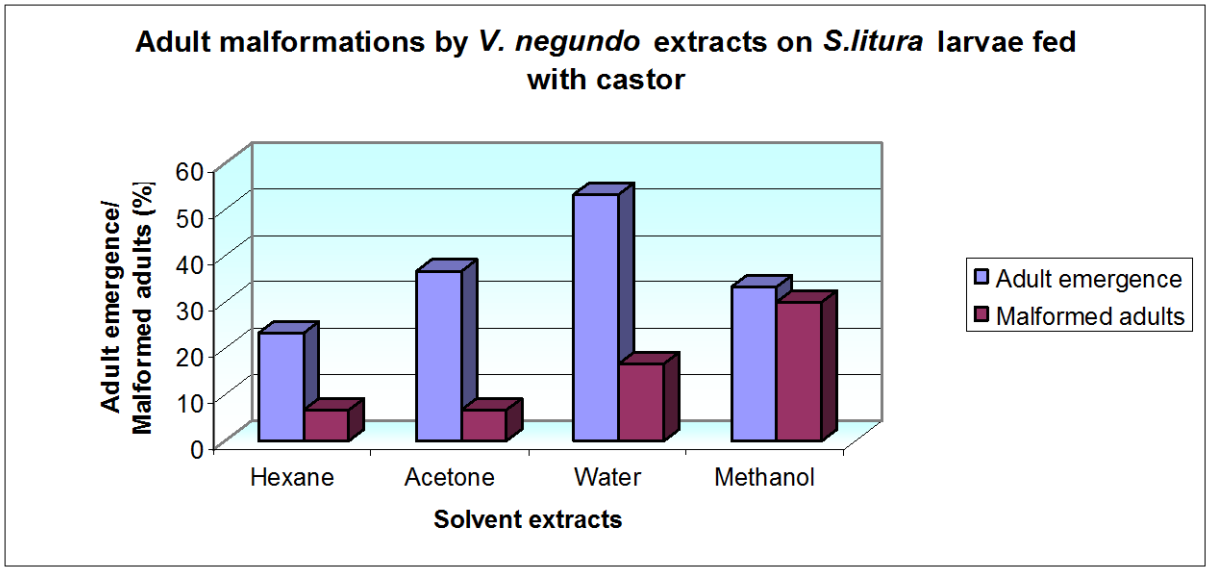

Pupal malformation by V. negundo extracts on S.litura larvae fed with castor
Pupation/Malformed pupation (%)
70
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts
Pupation
Malformed pupae

Fig. 31


Adult malformations by V. negundo extracts on S.litura larvae fed with castor
Adult emergence/ Malformed adults (%)
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts
Adult emergence
Malformed adults

extracts recorded lower pupation and adult emergence, higher malformed pupae and adults thus indicating the higher potency in causing morphogenic malformation. These findings are in consonance with the findings of Singh *et al*. (2005), where in they had reported that methanol extract of ginger at two per cent level recorded higher pupal malformations of 31 per cent deformed pupae. According to Vyas *et al*. (1999), plant extracts with custard apple (*Annona squamosa*) resulted in maximum deformed pupae. Experiments conducted by Senthilkumar, (2001) also revealed that acetone extracts of *Aristolochia bracteata* when treated to *S. litura* larvae caused deformed pupae and larval pupal intermediaries.

In semi-synthetic diet, lowest pupation (46.67%) was recorded by water extract, while highest malformed pupae and adults (26.67 and 30% respectively) were recorded by hexane extract at six per cent concentration. Lowest total adult emergence was observed in methanol extract (23%) (Figs. 32 & 33).

When the larvae were reared on banana, lowest pupation (40%) was recorded by methanol extract. Pupal malformation was highest in both hexane and methanol extracts (23.33% each). Adult emergence was lowest in hexane and acetone extracts (30% each). Highest adult deformation was recorded by methanol extract (30 %) (Figs.34 and 35).

When compared to all the hosts (castor, banana and semi-synthetic diet), insects reared on semi-synthetic diet recorded lower rate of malformations. This may be due to the higher nutritive status of food which enabled the insect to overcome the stress condition. But, even then at higher concentrations there was pupal and adult malformations.

In all the hosts, hexane and methanol extracts recorded lowest pupation, highest malformed pupae, lowest adult emergence and highest adult malformation indicating the efficacy of these extracts as potent insect growth regulators. So the present findings are agreeable with the experiments conducted by Singh *et al*. (2005) where in they had revealed that methanol extract of ginger resulted in

Fig. 32

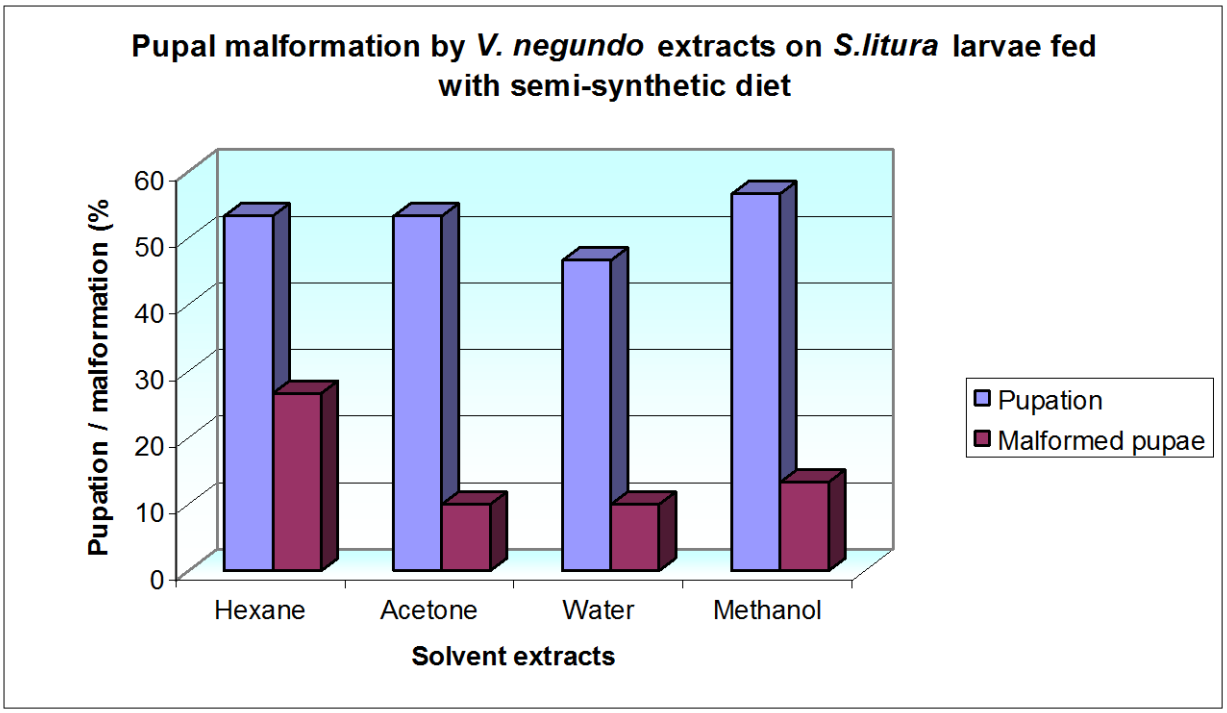

Pupal malformation by V. negundo extracts on S.litura larvae fed with semi-synthetic diet
Pupation / malformation (%
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts
Pupation
Malformed pupae

Fig. 33

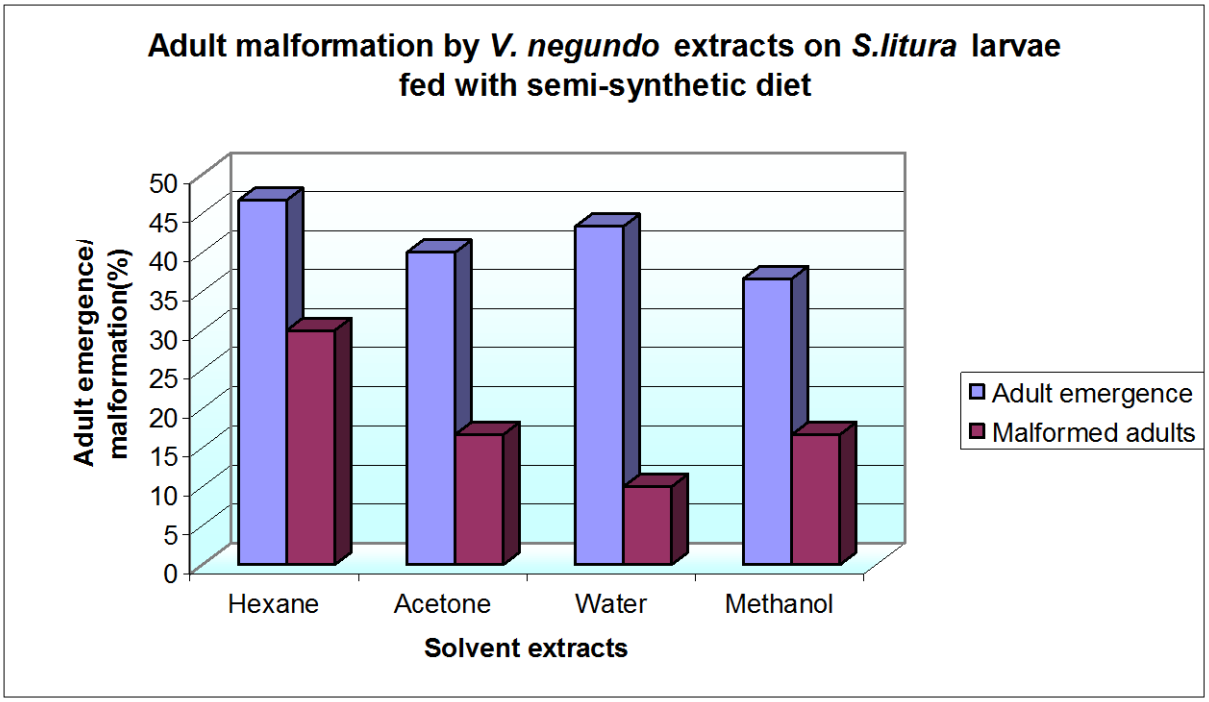

Adult malformation by V. negundo extracts on S.litura larvae fed with semi-synthetic diet
Adult emergence/ malformation(%)
50
45
40
35
30
25
20
15
10
5
0
Hexane
Acetone
Water
Methanol
Solvent extracts
Adult emergence
Malformed adults

Fig. 34

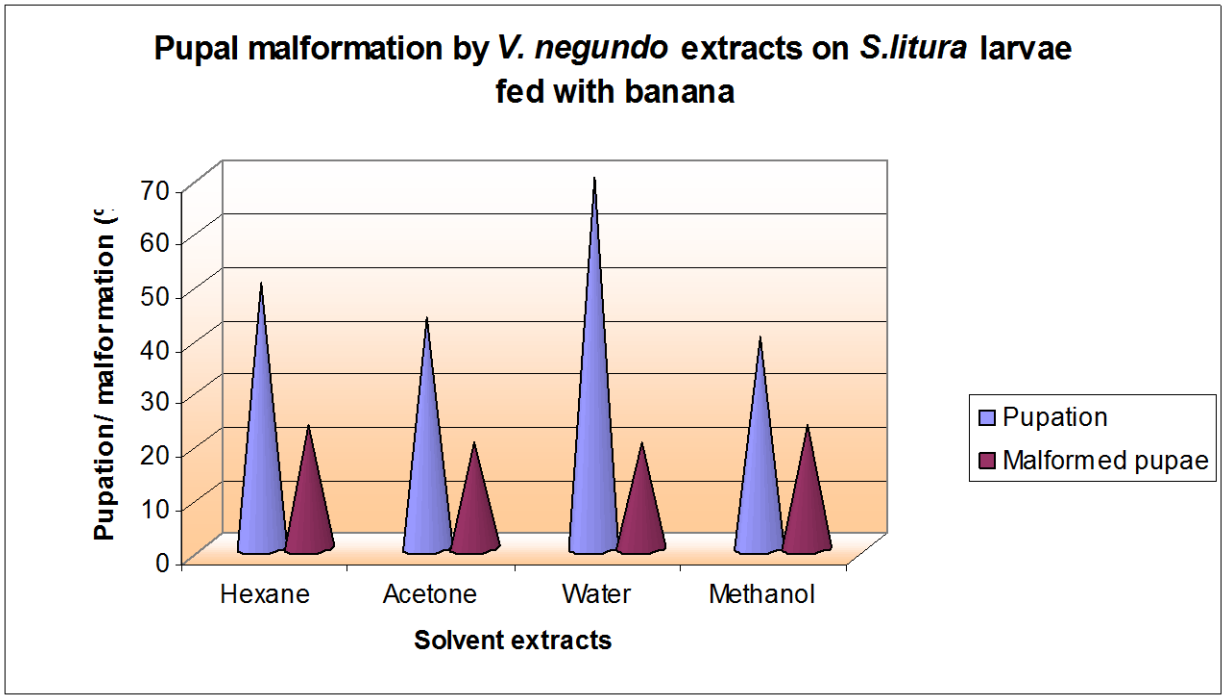
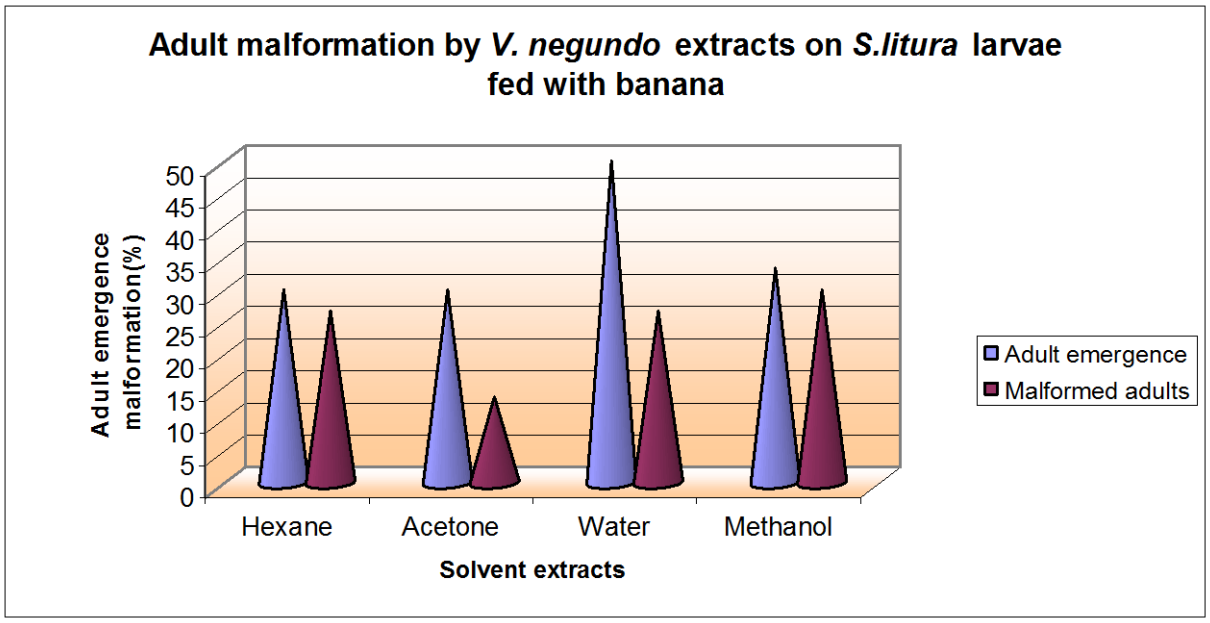

Pupal malformation by V. negundo extracts on S.litura larvae fed with banana
Pupation/ malformation (%)
70
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts
Pupation
Malformed pupae

Fig. 35


Adult malformation by V. negundo extracts on S.litura larvae fed with banana
Adult emergence malformation(%)
50
45
40
35
30
25
20
15
10
5
0
Hexane
Acetone
Water
Methanol
Solvent extracts
Adult emergence
Malformed adults

lowest adult emergence of 8.9 per cent and was followed by hexane extracts with only 13.33 per cent adult emergence.

Adult emergence showed a declining trend with increase in concentrations. From this it is clear that pupation is affected by the host food material and the activity of solvent extracts of *V. negundo* differs with the different host food materials on which *S. litura* larvae were reared. Experiments conducted by Singh and Rao, (2000) revealed that leaf ethanol extract *Ageratum conyzoides* prevented adult emergence upto 80 per cent.

It is evident from the present finding that host food material had an influence on the biology of *S. litura* by changing pupation, adult emergence etc. *V. negundo* solvent extracts recorded IGR activity by reduced pupation, adult emergence and malformations in pupae and adults and their effect varied with the host food materials on which *S. litura* larvae were reared. Huang *et al*. (2004) reported that ecdysone receptor protein was absent in azadirachtin treated *S. litura* and thus cause disruption to the growth and development of the insect. He also proposed that morphogenic malformations could be attributed to the disruption in the ecdysteroid titre and there by blocking of moulting process.

Furthur studies on protein metabolism of *V. negundo* treated insect is essential to find out the exact mode of action.

5.2.12.2. Morphogenic malformations as influenced by different solvent extracts of V. negundo against H. vigintioctopunctata

As evidenced from the Table 27 and Fig.36, it is clear that *V. negundo* solvent extracts are causing very less morphogenic malformations when compared to that applied against *S. litura*. Methanol extract (6%) caused lowest pupation (50%) as against highest pupation with hexane (1%). Acetone and water caused highest pupal malformation (20%) as against lowest in water extract. Lowest adult emergence was observed with water and methanol (43.33% each). Maximum deformed adults were obtained by hexane extract at six per cent level with a mean deformation of 13.33 per cent.

5.2.13. Longevity

Longevity denotes the life span of insects. As longevity is reduced, population of insect will also be reduced thereby lowering the economic damage to crops.

5.2.13.1. Effect of different solvent extracts of V. negundo against longevity of S. litura

An experiment was laid out as per 3.4.3. in order to assess the effect of different solvent extracts of *V. negundo* on longevity of *S. litura* adult moths. Results of this experiment are presented in Table 28 and Fig.37.

There was no significant difference in the longevity of adult moths in the control treatment (solvents alone) indicating that solvents alone had no influence on the longevity of adult moths of *S. litura*. Longevity of *S. litura* was found to decrease with increase in concentration of *V. negundo* extract. *V. negundo* extract significantly reduced life span of *S. litura*. The solvents alone showed no significant difference on the longevity of *S. litura*. Acetone extract (6%) and water extract (4%) caused lowest longevity of 0.67 days. All the solvent extracts at six per cent concentration resulted in more than 80 per cent reduction in longevity (Fig.37).

From this study, it could be concluded that solvent extracts of methanol and hexane can effectively suppress the pest population by reducing adult longevity even at lower concentrations itself. At higher concentrations, acetone extracts also reduced longevity of adults to a greater extend. More experiments are to be conducted to establish the efficiency of these extracts at field level.

5.2.13.2. Effect of different solvent extracts of V. negundo against longevity of H. vigintioctopunctata

In case of epilachna beetles, adults recorded life span of 16-17 days. Longevity of *H. vigintioctopunctata* also showed a decrease by the application of increasing concentrations of *V. negundo* extracts. All the solvent extracts

Fig. 36

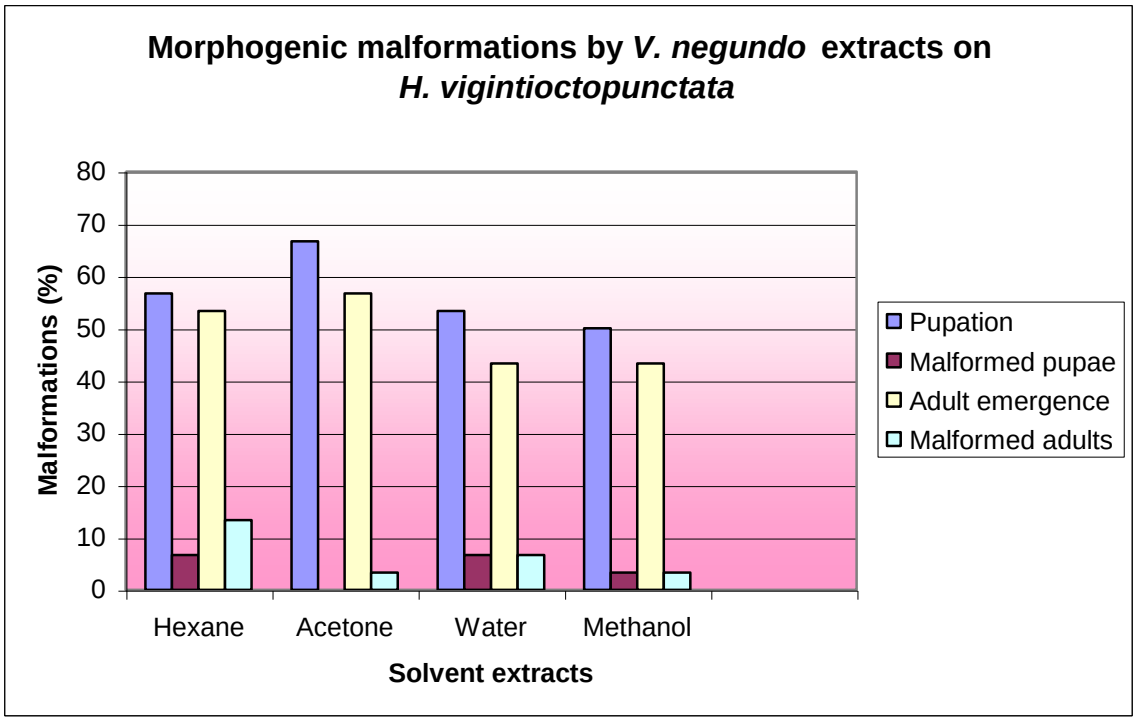
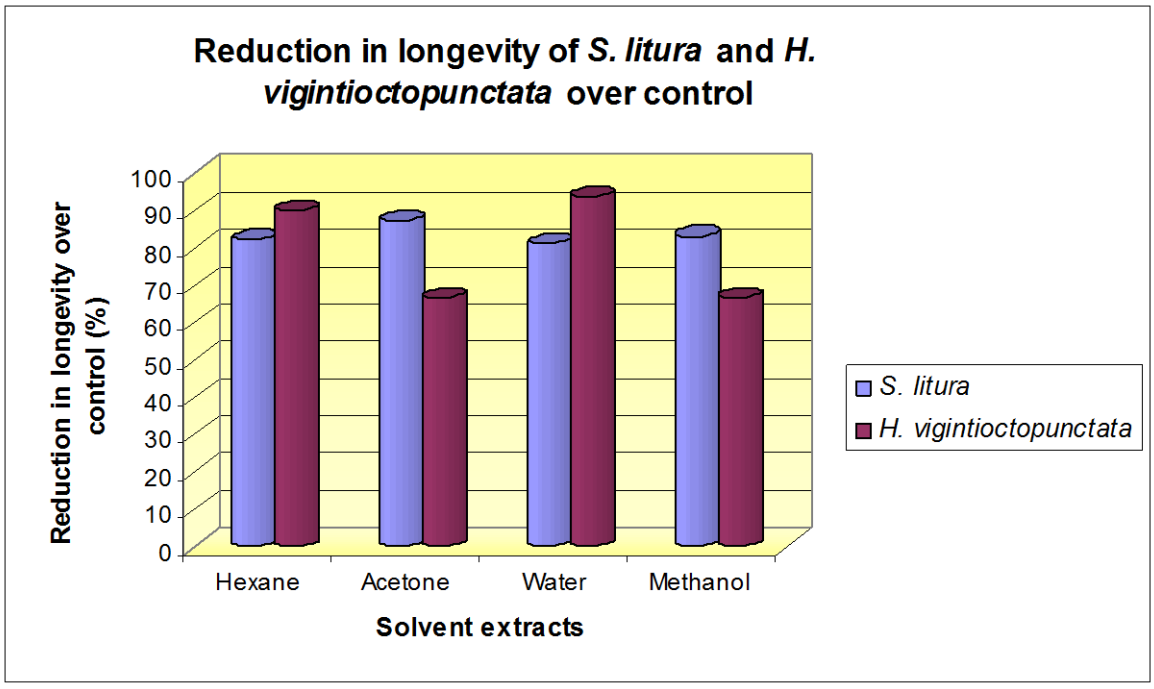

Morphogenic malformations by V. negundo extracts on H. vigintioctopunctata
Malformations (%)
80
70
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts
Pupation
Malformed pupae
Adult emergence
Malformed adults

Fig. 37


Reduction in longevity of S. litura and H. vigintioctopunctata over control
Reduction in longevity over control (%)
100
90
80
70
60
50
40
30
20
10
0
Hexane
Acetone
Water
Methanol
Solvent extracts
S. litura
H. vigintioctopunctata

significantly reduced life span of adult beetles. Hexane and water extracts (6%) resulted in lowest longevity (1.0 and 1.33 days) as against methanol extract (6%) causing highest longevity (5.30 days). The growth inhibition effect of *V. negundo* is thus evident with regard to longevity also. Hexane and water extract of *V. negundo* caused maximum reduction in longevity (90.18 and 93.75% respectively) (Fig.37).

5.2.14. Fecundity

5.2.14.1. Effect of different solvent extracts of V. negundo against fecundity of S. litura

Fecundity denotes the capacity of an adult female to lay eggs. Since it gives a direct measure of population/generation of the insect, study on the effect of different solvent extracts on the fecundity of insects is very important. Results of this experiment are presented in Table 29 and Fig.38.

Adult moths in control treatment recorded an average of 546.33 eggs. All the solvent extracts significantly reduced fecundity even at lower concentrations itself. There was no significant difference among the different solvents (control) of hexane, acetone, water and methanol. No eggs were laid with hexane (4 and 6%), acetone (6%) and water (6%) extracts (cent per cent reduction in fecundity) thus proving the efficacy of solvent extracts of *V. negundo* as a potent inhibitor of egg laying. Nathan *et al*. (2005) reported that fecundity of *Nilaparvata lugens* Stal. was significantly reduced in azadirachtin (AZA) treatments compared to control. Histological study of ovary sections revealed abnormalities in follicular epithelial cells due to AZA treatment. Similar observations were also made by Nathan *et al*. (2009) where they reported that when the pure triterpenes of *Dysoxylum* when applied against rice leaf folder larvae, fecundity was reduced showing a decreasing trend with increase in concentration. At highest concentration of 25 ppm, lowest fecundity of 7 eggs (3 % of control) was observed. Reduction in fecundity may be due to the disruption in the neuroendocrine centre of moulting in insects (Rembold and Sieber, 1981; Kubo and Klocke 1986; Rembold, 1988; Singh, 2003 ; Nathan *et al*., 2009).

Fig. 38

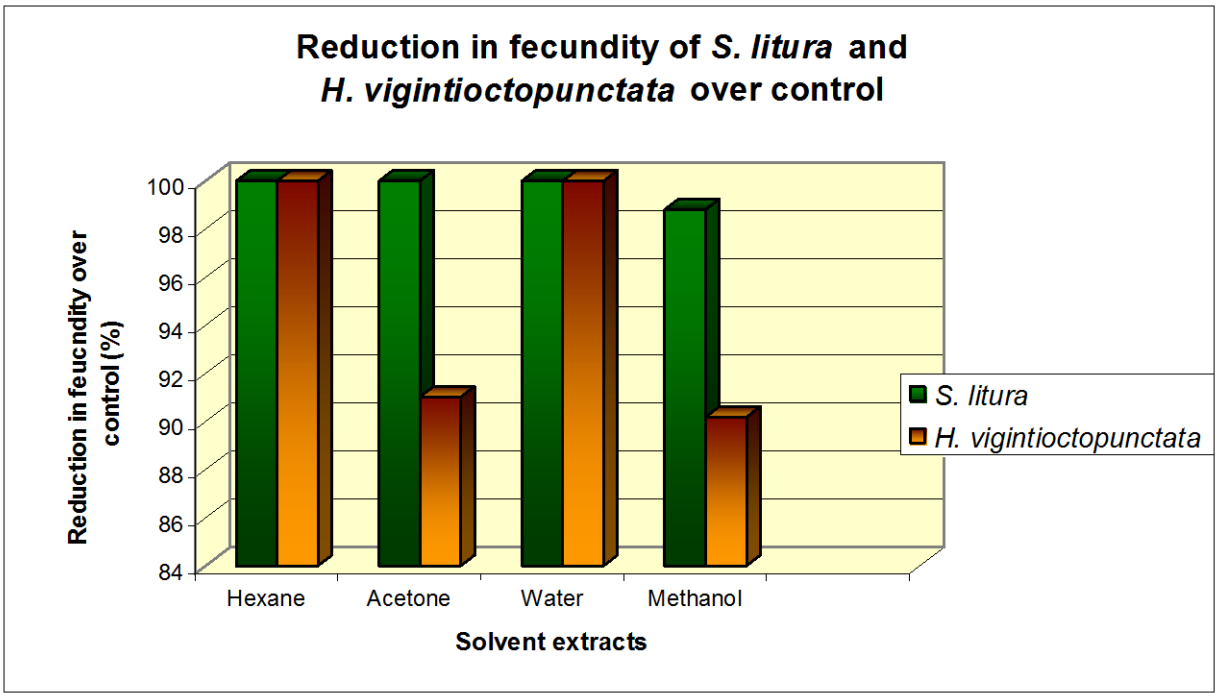
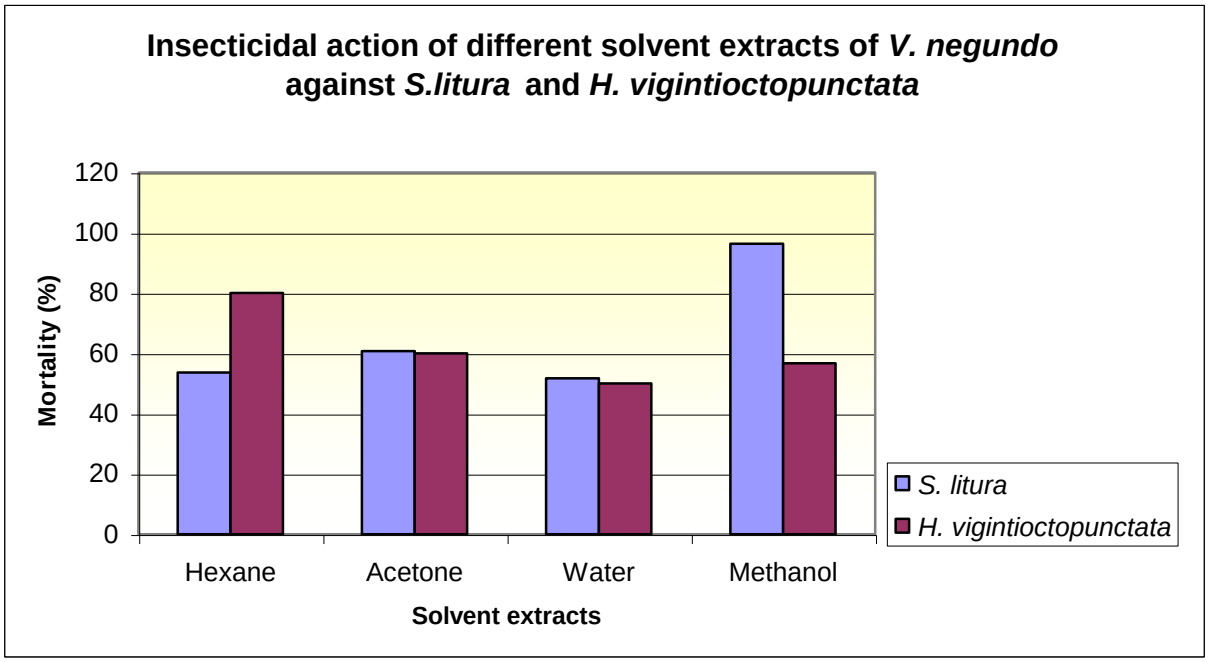

Reduction in fecundity of S. litura and H. vigintioctopunctata over control
Reduction in feucndity over control (%)
100
98
96
94
92
90
88
86
84
Hexane
Acetone
Water
Methanol
Solvent extracts
S. litura
H. vigintioctopunctata

Fig. 39


Insecticidal action of different solvent extracts of V. negundo against S.litura and H. vigintioctopunctata
Mortality (%)
120
100
80
60
40
20
0
Hexane
Acetone
Water
Methanol
Solvent extracts
S. litura
H. vigintioctopunctata

The present study indicates possible interference of these solvent extracts in the reproductive functioning of insects. This is in confirmation with the findings of Huang *et al*. (2004) where in a reduction in fecundity was observed in azadirachtin treated *S. litura* larvae. They proposed that reproductive reduction in azadirachtin treated insects is correlated with the changes of protein metabolism in female pupae.

By reducing fecundity forecoming population can be reduced to a greater extend and thereby reduce the economic damage and thus crop loss could be minimized.

5.2.14.2. Effect of different solvent extracts of V. negundo against fecundity of H. vigintioctopunctata

Here also, *V. negundo* extracts significant reduction in the number of eggs laid as against control treatments where only solvents are used. Hexane and aqueous extracts (6%) caused complete inhibition of egg laying of *H. vigintioctopunctata*. This is in accordance with the findings of Sahayaraj *et al*. (2006), in which they reported that root ethanol extracts of *Pedalium murex* reduced ovipositional time and number of eggs laid in case of *Dysdercus cingulatus* (Fab.). Studies conducted by Rao and Subrahmanyam, 1987 reported that fecundity in azadirachtin treated individuals of *Achea janata* was reduced to half than that of untreated individuals. The female adults of *S. litura* which emerged from treated larvae had prolapsed anal brushes.

5.3. MEDIAN LETHAL DOSE (LD_{50})

5.3.1. Effect of different solvent extracts of *V. negundo* against LD_{50} of *S. litura*

In case of *S. litura*, methanol extract was more toxic and efficient in causing mortality and it recorded lowest LD_{50} of 423 ppm. Highest LD_{50} was recorded by aqueous extract with a value of 590 ppm. Hexane extract also recorded relatively higher LD_{50} of 562 ppm while acetone extracts recorded LD_{50} of 517 ppm (Table 30).

5.3.2. Effect of different solvent extracts of *V. negundo* against LD_{50} of *H. vigintioctopunctata*

When the solvent extracts were tested against Epilachna beetles, extracts with hexane recorded highest toxicity to *H. vigintioctopunctata* with an LD_{50} of 348 ppm. Water extracts recorded highest LD_{50} of 666 ppm indicating its lowest toxicity. Methanol extracts also recorded comparatively higher LD_{50} of 507 ppm when compared to the extracts with hexane and acetone (Table 31). This is in confirmation with the findings of Singh and Singh, (2008) in which they reported cent per cent mortality of *H. vigintioctopunctata* when treated with four per cent concentration of benzene and acetone extracts of *Trichilia connaroides* Bentilizen. Methanol, ethyl acetate and benzene extracts were also equally effective in causing mortality. Similar results were also reported by Rahman and Talukder (2006) where they reported that among the different plant extracts tested for their toxicity against pulse beetle, leaf acetone extract of nishinda (*V. negundo*) was the most toxic one with lowest LC_{50}.

5.4. INSECTICIDAL ACTION

To determine insecticidal action of different solvent extracts of *V. negundo* an experiment was laid out as described in 3.6.5. Results of this study are presented in Table 32.

5.4.1. Effect of different solvent extracts of *V. negundo* on insecticidal action against *S. litura*

Third instar larvae of *S. litura* were treated with different solvent extracts of *V. negundo* at different concentrations. Mortality count after 120 hrs was taken, results are analysed and presented in Table 32 and Fig.39.

Among all the four solvent extracts, methanol extract at six per cent level recorded significantly highest mortality in *S. litura* (96.4%) (Fig.39). Experiments conducted by Singh *et al*. (2005) also reveled insecticidal properties of methanol extracts of ginger in which they got 78.9 per cent mortality when the larvae of *Earias vittella* were treated with 10 per cent concentration of methanol

extract of ginger. In a field experiment conducted to test the efficiency of different plant extracts, alcoholic extracts of *V. negundo* at two per cent concentration resulted in higher mortality of 74.26 per cent against third instar larvae of *Spilosoma obliqua* (Dubey *et al.*, 2004). Similar were the findings of Gautham *et al*. (2003) in which they had reported maximum insecticidal action of methanolic extracts of *Saussurea heteromala* (a member from Asteraceae family) against *S. obliqua* with a mean per cent mortality of 65.3

Acetone extract at six per cent level also recorded higher mortality of 60.7 per cent. This is in consonance with the findings of Saradamma (1989), in which she had reported that acetone extracts of *V. negundo* resulted in cent per cent mortality of *S. litura*. Similar findings were also made by Kalavathy *et al*. (1991) where the insecticidal activity of acetone extracts of *V. negundo* extracts against *E. vitella* was reported. Dayrit *et al*. (1995) had recorded that topical application of volatile oils of *V. negundo* caused 91 per cent mortality in third instar larvae of *S. litura*. Devanand and Rani (2008) reported that crude acetone extracts of *Tectona grandis, Mangifera indica* and *Momordica charantia* produced higher mortality (> 80 %) to *S. litura* and *A. janata*

Hexane and water extracts recorded lowest mortalities of 53.6 and 51.7 per cent respectively at six per cent level.

5.4.2. Effect of different solvent extracts of *V. negundo* on insecticidal action against *H. vigintioctopunctata*

In the case of *H. vigintioctopunctata*, hexane extract (6%) caused highest mortality (80%). This was followed by acetone extract with 60 per cent mortality at six per cent level (Table 32). Water and methanol extracts (6%) showed no significant difference in mortality to *H. vigintioctopunctata*. Insecticidal action of acetone extracs of *V. negundo* was reported earlier by Moore *et al*. (1989) where it was stated that acetone extracts of *V. negundo* caused cent per cent mortality of *H. vigintioctopunctata*.

In case of *S. litura*, the most effective and toxic solvent extract was methanol at six per cent concentration (96.40%) while against *H.*

vigintioctopunctata, hexane extract at six per cent recorded highest mortality proving its highest toxicity (Fig.39). Hexane extract recorded lowest mortality against *S. litura* and was on par with the water extract at six per cent level (51.70%), while the same hexane extract recorded highest mortality against *H. vigintioctopunctata*. From this it is clear that the insecticidal action of solvent extracts of *V. negundo* differs with the test insects used for the study.

5.5. RELATIVE EFFICIENCY OF *V. NEGUNDO* EXTRACTS AND MICROBIALS ON *S. LITURA* AND *H. VIGINTIOCTOPUNCTATA*

5.5.1. Compatibility of entomopathogens with botanicals and insecticides

Compatibility of botanicals and chemical insecticides are of very important in improving the efficacy of entomopathogens in pest management systems. Joint action of entomopathogens with botanicals/ insecticides will enhance the activity of entomopathogens.

5.5.1.1. Effect of V. negundo extracts on Metarhizium anisopliae

An experiment was designed to determine the inhibitory action of different insecticides and solvent extracts of *V. negundo* on the growth of *M. anisopliae*. Per cent inhibition over control was determined in different treatment as per 3.6.1 and the results are presented in Table 33 and Fig.40.

Methanol extract of *V. negundo*, azadirachtin and quinalphos showed equally higher inhibition (71.06 to 54.90 %) of *M. anisopliae* indicating their incompatibility with *M. anisopliae*. The fungicidal action of quinalphos was earlier reported by Kalpana (1992) against *Pyricularia oryzae* and *Colletotrichum gloeosporioides* (Deepthy, 2004). The present finding on fungal growth inhibition of azadirachtins in agreement with Aguda and Rombach, (1986) who had reported that neem oil at 5 per cent concentration inhibited germination and sporulation of *M. anisopliae.*

Hexane, acetone and aqueous extracts inhibited growth of *M. anisopliae* to a lesser extent of 3.04 to 19.58 per cent. Levin (2005) reported that aqueous extract of *V. negundo* resulted in lowest inhibition of *M. anisopliae*. It is thus proved

Fig. 40

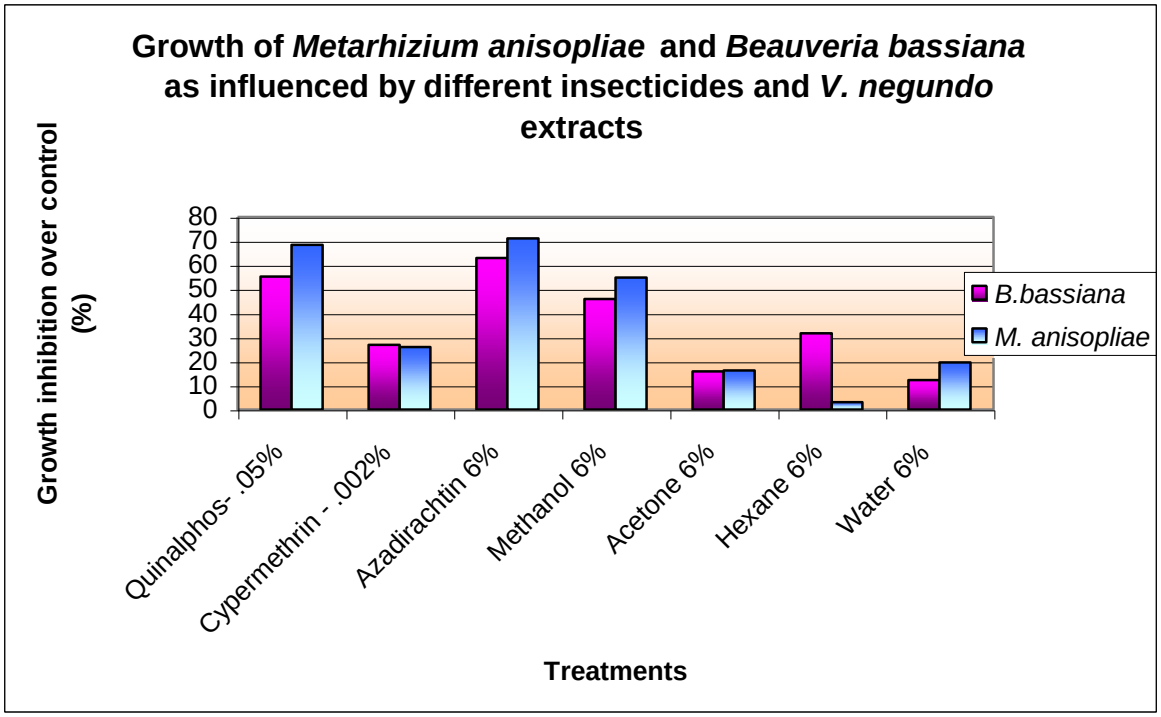
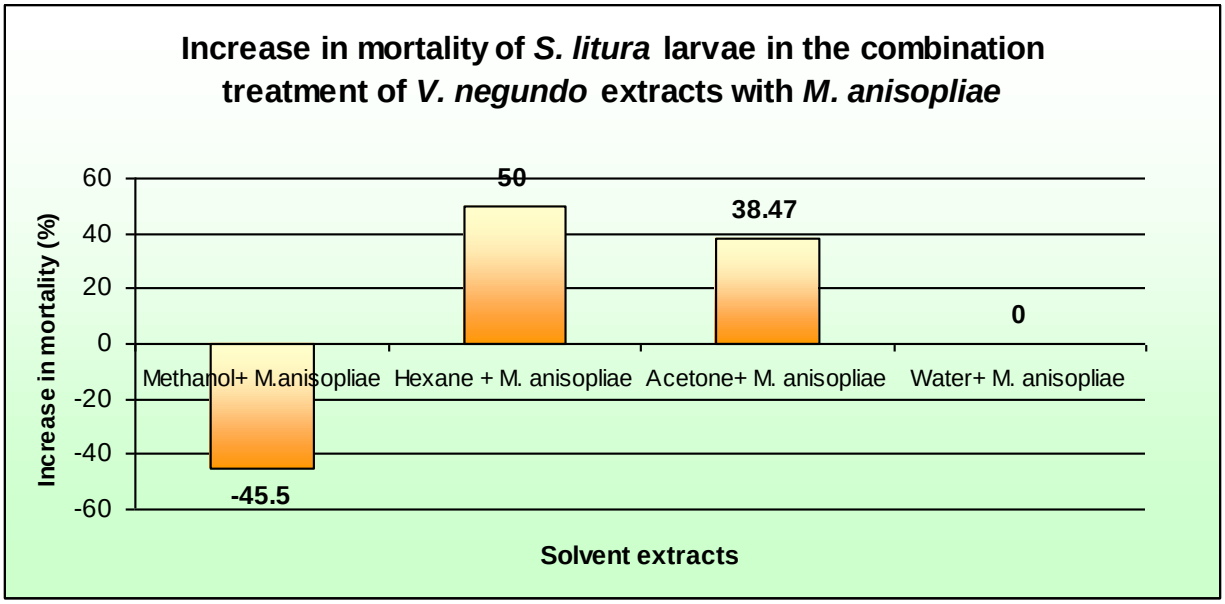

Growth of Metarhizium anisopliae and Beauveria bassiana as influenced by different insecticides and V. negundo extracts
Growth inhibition over control (%)
80
70
60
50
40
30
20
10
0
B.bassiana
M. anisopliae
Quinalphos- .05%
Cypermethrin - .002%
Azadirachtin 6%
Methanol 6%
Acetone 6%
Hexane 6%
Water 6%
Treatments

Fig. 41


Increase in mortality of S. litura larvae in the combination treatment of V. negundo extracts with M. anisopliae
Increase in mortality (%)
60
40
20
0
-20
-40
-60
50
38.47
0
-45.5
Methanol+ M.anisopliae
Hexane + M. anisopliae
Acetone+ M. anisopliae
Water+ M. anisopliae
Solvent extracts

these extracts are compatible with *M. anisopliae* for the management of pests. Azadirachtin, quinalphos and methanol extract of *V. negundo* are incompatible with *M. anisopliae*.

5.5.1.2. Effect of V. negundo extracts on Beauveria bassiana

Poison food technique was conducted to determine the detrimental effect of certain insecticides and botanicals on the growth of *B. bassiana*. It is evident that, as in the case of *M. anisopliae*, azadirachtin at 0.03 per cent inhibited growth of *B. bassiana* (63.01%) (Fig.40). Similar results were also obtained by Devaprasad *et al.* (1989). According to them, neem oil and neem seed kernel extracts were highly deleterious to the spore germination of *B. bassiana.* Insecticide quinalphos showed fungitoxic effect against *B. bassiana* with a mean per cent inhibition of 52.8 per cent. According to Malo, (1993), insecticides *viz*., fenitrothion, primiphos methyl, endosulfan and dicrotophos inhibited germination and growth of *B. bassiana.* When compared to *M. anisopliae*, methanol extract recorded less than 50 per cent inhibition on the growth of *B. bassiana*. According to Vyas *et al*. (1992), extracts of certain botanicals such as *A. indica*, *Pongania glabra* and *Madhuca indica* inhibited growth of *B. bassiana*. Hexane, acetone and water extracts of *V. negundo* recorded lower per cent inhibition and were considered compatible with the fungus. In an experiment conducted by Levin (2005), different plant extracts were tested for their compatibility with the fungus *B. bassiana*. Among the different extracted tested, all the plant extracts (*A. calanus, A. paniculata* and *V. negundo*) caused more than 50 per cent inhibition.

5.5.1.3. Compatibility of B. thuringiensis with insecticides and botanicals

Compatibility of *B. thuringiensis* with insecticides and botanicals were studied by filter paper method. None of the treatments developed inhibition zone and thus it could be concluded that all the treatments were highly compatible with *B. thuringiensis*. Antibacterial activity of *V. rotundifolia* against methicillin resistant *Staphylococcus aureus* was reported by Kawazoe *et al*. (2000). Eventhough *V. negundo* extracts is having antibiotic properties against

Staphylococcus aureus, it is not having any inhibition against the entomopathogenic bacteria, *B. thuringiensis*.

5.5.2. Joint action of entomopathogens with different solvent extracts of *V. negundo*

Pest management using entomopathogens are ecologically non disruptive, safe and specific, but their delayed action due to prolonged incubation period, shorter half life period, insufficient control against grown up stages of the pest species necessitated the need for integration with other methods for faster killing. Plant species with sources of biologically active chemicals were studied extensively in combination with bio pesticides. Successful combination treatment of entomopathogens with botanicals / insecticides will result in synergestic action and also help to reduce the mean lethal time (LT_{50}) of microbials/botanicals. Hence integrating microbials with botanicals / insecticides is of very important.

5.5.2.1. Combined action of different solvent extracts of V. negundo and M. anisopliae against S. litura

Results of this experiment are presented in Tables 34 and 36 snd Figs. 41 to 42.

V. negundo extract in methanol caused highest mortality (73.33 %) at 10 days after treatment (DAT) and hexane extract resulted in lowest mortality (40.0%). But, the combination of methanol extract with *M. anisopliae* reduced the mortality to 40 per cent. Hexane and acetone extracts of *V. negundo* in combination with *M. anisopliae* increased the mortality from 40 to 60 per cent resulting in 50 per cent increase in mortality when compared to hexane alone treatment (Fig.41). Combination of aqueous extract with *M. anisopliae* caused no change in mortality. But the lethal time taken for 50 per cent mortality was reduced from 9.15 days to 8.7 days. Lowest LT_{50} was recorded by methanol extract (4.65days) and the highest by hexane extract (12.09days). But in combination treatment of hexane extract with *M. anisopliae* LT_{50} was reduced to 7.10 days with 41.27 per cent reduction in LT_{50} when compared to hexane alone treatment (Fig.42). Acetone extract with *M. anisopliae* also reduced LT_{50} to 7.38

days, while it was 11.29 days when applied alone. In an experiment conducted by Levin (2005) revealed that in the laboratory bioassay of entomopathogenic fungi and plant extract combinations, treatment combination of *M. anisopliae* with *V. negundo* water extracts recorded higher mortality. In the previous experiment of compatibility of different insecticides and solvent extracts of *V. negundo* on the growth of *M. anisopliae*, it was found that methanol extract was inhibiting the growth of fungus with a mean per cent inhibition of 54.90. Hence in combination treatment of *M. anisopliae* with methanol extract, mortality of larvae was reduced with only upto 40 per cent and no synergestic action was observed in the treatment combination of *M. anisopliae* with methanol extract.

On the tenth day, treatment with *M. anisopliae* alone recorded a mean mortality of 63.33 per cent. Hexane and acetone extracts when used alone resulted in lower mortalities of 40 and 43.33 per cent respectively. But in combination treatments with *M. anisopliae* higher mortalities of 60 per cent were recorded indicating subadditive synergism. Among the different combinations of botanicals with *Nomorea riley Samson* ($2x10^{11}$ conidia\ha) +NSKE (5 per cent) proved to be as effective as recommended insecticides in reducing larval incidence of *S. litura* (Patil *et al.,* 2003).

All the solvent extracts except methanol recorded longer time for 50 per cent mortality. Hexane extract when used alone required 12.09 days for causing mortality to 50 per cent population of *S. litura* larvae while in the combination treatment with *M. anisopliae*, lethal time required to cause 50 per cent mortality was reduced to 7.10 days. Similar results were also observed in case of acetone and water extracts in which mean LT_{50} obtained were 11.29 and 10.63 days respectively. But, in combination treatments with *M. anisopliae*, lethal time got reduced to 7.38 and 8.70 days respectively resulting in 34.63 and 4.92 per cent reduction in LD_{50} when compared to the treatments in which they are applied alone (Fig.42). All these combinations exhibited temporal synergism. When the fungus *M. anisopliae* were used alone, LT_{50} value recorded was 9.37 days and the lethal time period gets shortened in all the combination treatments except combination with methanol extract, showing temporal synergism. From this

Fig. 42

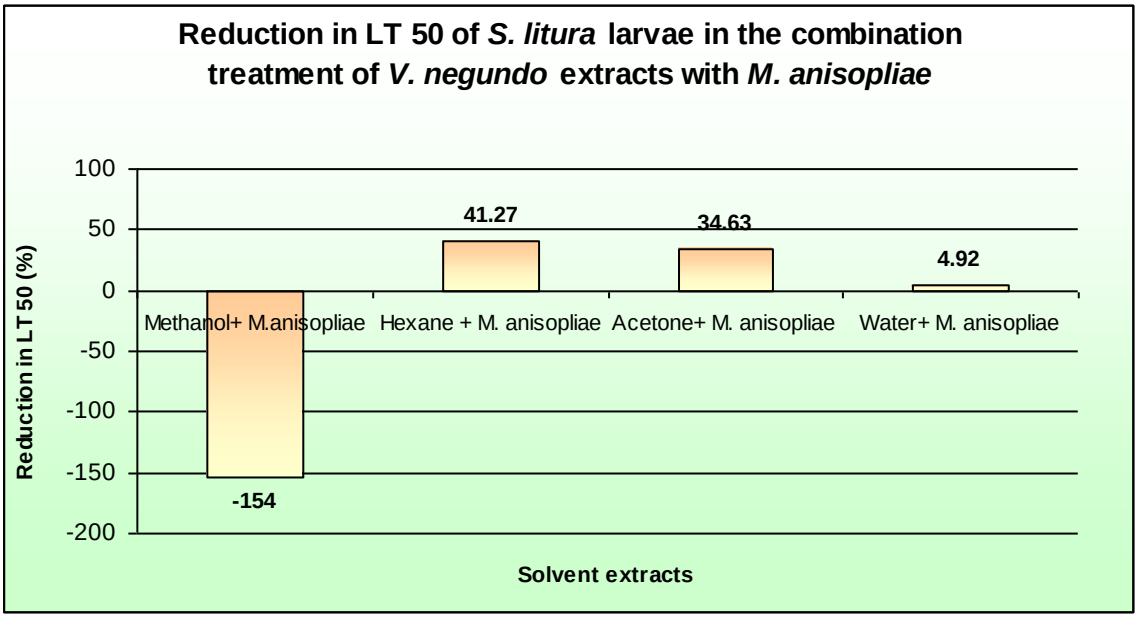
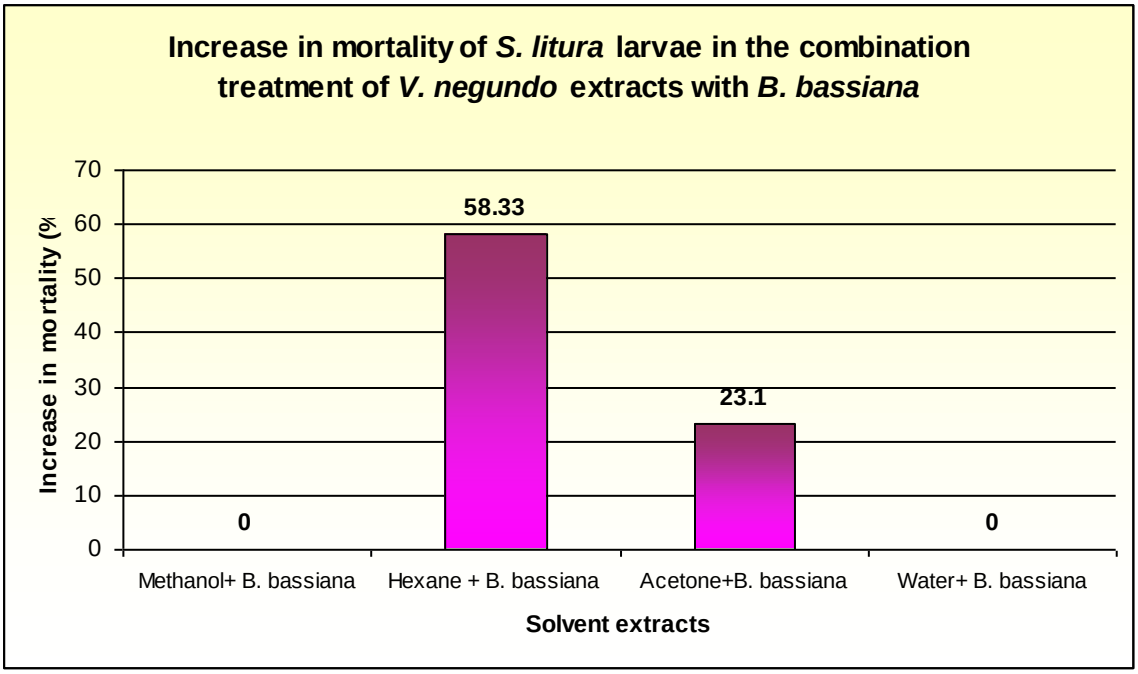

Reduction in LT 50 of S. litura larvae in the combination treatment of V. negundo extracts with M. anisopliae
100
50
0
-50
-100
-150
-200
Reduction in LT 50 (%)
41.27
34.63
4.92
-154
Methanol+ M.anisopliae
Hexane + M. anisopliae
Acetone+ M. anisopliae
Water+ M. anisopliae
Solvent extracts

Fig. 43


Increase in mortality of S. litura larvae in the combination treatment of V. negundo extracts with B. bassiana
70
60
50
40
30
20
10
0
Increase in mortality (%
58.33
23.1
0
0
Methanol+ B. bassiana
Hexane + B. bassiana
Acetone+B. bassiana
Water+ B. bassiana
Solvent extracts

experiment it is concluded that in the treatment combination of *M. anisopliae* with all the solvent extracts (except methanol extract) reduced the feeding period of larvae to a greater extend and thus reduce the damage considerably. When the larvae were treated with plant extracts, a stress condition will develop in the larval body, which would predispose fungal infection.

5.5.2.2. Combined action of different solvent extracts of V. negundo and B. bassiana against S. litura

An experiment was done to determine the combined efficacy of *B. bassiana* and *V. negundo* solvent extracts and the results are presented in the Tables 36 and 37 and Figs.43 and 44.

Upto seventh day, there was no mortality in the treatment with *B. bassiana* alone. At the same time, all the treatment combinations of *B. bassiana* with different solvent extracts of *V. negundo* recorded higher mortalities. Among the different treatment combinations, combination of *B. bassiana* with methanol extract resulted in higher mortality of 63.33 per cent. Higher insecticidal action of methanol extract combined with the action of *B. basiana* resulted in highest mortality. Mortality percentage observed in treatment combination of hexane, acetone and water extracts with *B. bassiana* resulted in mean per cent mortalities of 40, 40 and 53.33 per cent respectively while at the same time when these extracts were treated alone recorded only 20, 30 and 20 per cent mortalities indicating synergestic effect of treatment combinations (Table 35). This is in confirmation with the findings of Babu *et al*. 2001, where highest mortality of 96 per cent against *S. litura* larvae were obtained in the combination treatment of *B. bassiana* with NSKE.

Combination treatments of hexane and acetone extracts with *B. bassiana* resulted in higher mortalities (58.33 and 23.10% increase) when compared to the treatments in which they are applied alone (Fig.43).

Lowest LT_{50} was observed in the treatment with methanol extract alone with 4.65 days. When *B. bassiana* was applied alone, higher LT_{50} of 9.05 days was recorded while in combination studies, there was a reduction in LT_{50} upto

Fig. 44

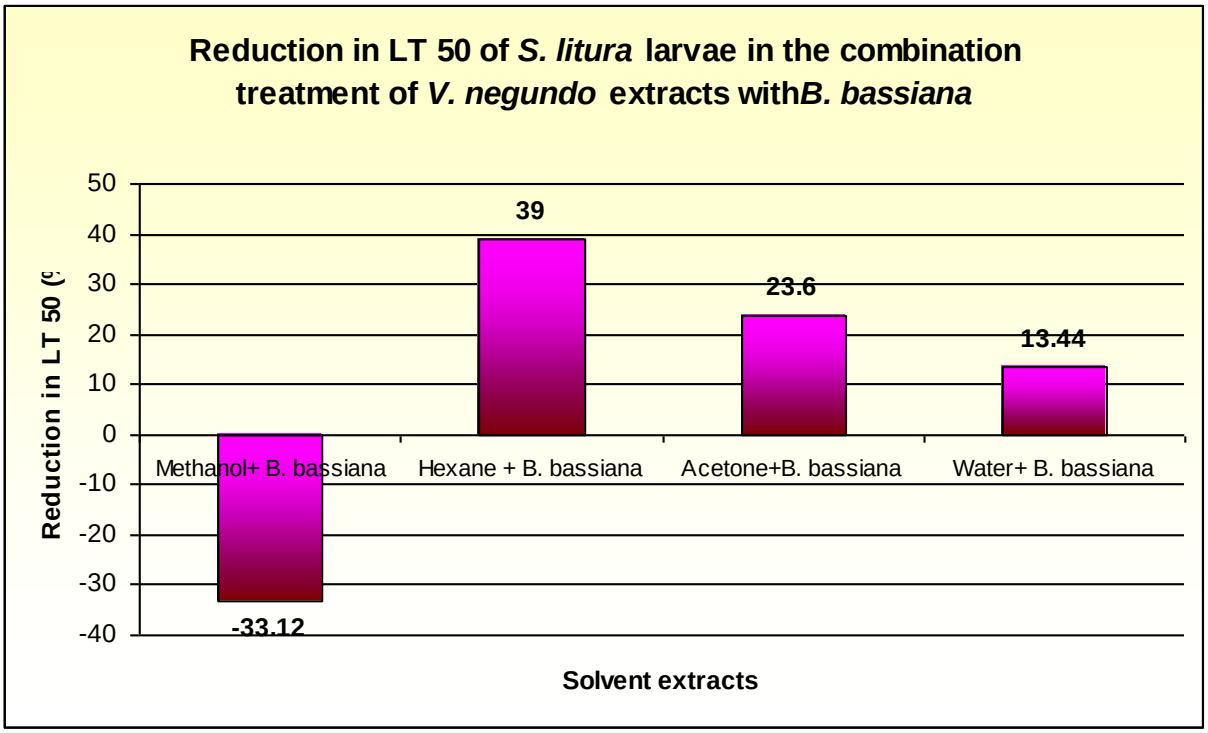
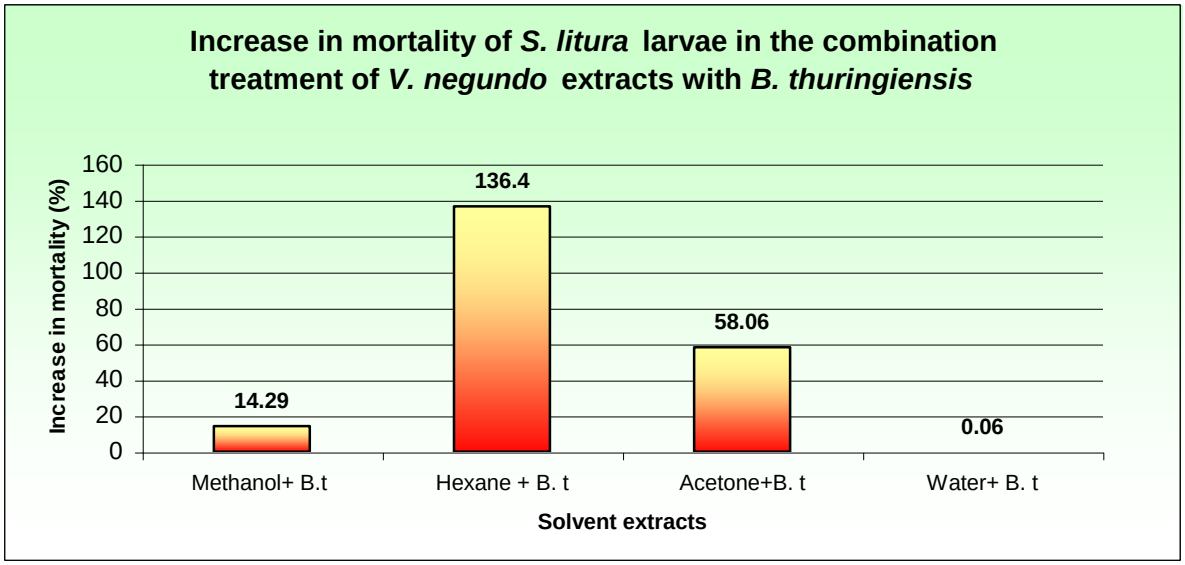

Reduction in LT 50 of S. litura larvae in the combination treatment of V. negundo extracts withB. bassiana
Reduction in LT 50 (
50
40
30
20
10
0
-10
-20
-30
-40
39
23.6
13.44
-33.12
Methanol+ B. bassiana
Hexane + B. bassiana
Acetone+B. bassiana
Water+ B. bassiana
Solvent extracts

Fig. 45


Increase in mortality of S. litura larvae in the combination treatment of V. negundo extracts with B. thuringiensis
Increase in mortality (%)
160
140
120
100
80
60
40
20
0
136.4
58.06
14.29
0.06
Methanol+ B.t
Hexane + B. t
Acetone+B. t
Water+ B. t
Solvent extracts

6.19, 7.30, 8.63 and 7.92 days when used in combination with methanol, hexane, acetone and water extracts. When the insects were treated with hexane extracts alone, the LT_{50} obtained was 12.09 days

whereas in the combination treatment with fungus *B. bassiana*, LT_{50} was reduced to 7.93 days with 39 per cent reduction in LT_{50}. Same trend was observed in case of acetone and water extracts with highest LT_{50} values of 11.29 and 9.15 days when used alone and there was a reduction in LT_{50} upto 8.63 and 7.92 days (23.6 and 13.44% reduction in LT_{50}) in case of combination treatments of the same extracts with *B. bassiana*. Water extracts produced an LT_{50} of 9.15 days and it was reduced upto 7.94 days in combination treatment with *B. bassiana* (Fig.44). All these combinations in which the LT_{50} values were lowered are recorded under temporal synergism (Table 36).

Interaction effect of combining *B. bassiana* with sub lethal doses of different plant extracts of *V. negundo* are presented in Table 37. Treatment combinations of methanol and water extracts with *B. bassiana* resulted in supplemental synergism. Sub additive synergism was observed in combination treatments of hexane and acetone extracts with *B. bassiana* (Table 37).

5.5.2.3. Combined action of different solvent extracts of V. negundo and B. thuringiensis against S. litura

Data on the combination studies of *B. thuringiensis* and solvent extracts of *V. negundo* are presented in Tables 38 and 39 and Figs.45 and 46.

In case of treatment with *B. thuringiensis* alone, mortality of the larvae started only from the second day onwards with 10 per cent mortality, while at the same time, treatment combinations of *B. thuringiensis* with methanol and hexane extracts recorded mortality on the first day of treatment itself. Upto 8 th day after treatment *B. thuringiensis* recorded a mortality of 53.3 per cent. At the same time treatment combination of *B. thuringiensis* with hexane extract recorded highest mortality of 90 per cent. Treatment combinations of *B. thuringiensis* with methanol and water extracts of *V. negundo* also recorded higher mortalities of 76.67 and 66.67 per cent respectively. Maximum increase in mortality was observed in combination treatment of hexane extract with *B. thuringiensis*

(136.4% increase over hexane alone treatment). Combination treatments of acetone and methanol extracts with *B. thuringiensis* also resulted in higher mortalities (58.06 and 14.29% increase), when compared to the treatments in which they are applied alone (Fig.45). Similar results were recorded by Levin, (2005) where in highest mortality was observed when *B. thuringiensis* was used in combination with *V. negundo* aqueous extracts. In all the treatment combinations, it was found that the action of *B. thuringiensis* was enhanced by the botanical extracts. Experiments done by Justin *et al*. (1987) revealed that *B. thuringiensis* when used in combination with neem seed kernel extract (5%) against third instar larvae of *Plutella xylostella* , LC_{50} value was reduced by 1.9 times. Enhancing effects of plant extracts like *Azadirachta indica, Ayapana triplinervis* and *Lantana camera* against *P. xylostella* and *Crocidolomia binotalis* when used in combination with *B. thuringiensis* was reported by Facknath, (1999). Works done by Chatterjee and Senapati (2000) also reported that joint spraying of *B. thuringiensis* (0.05%) and azdirachtin 1500 ppm (0.15%) recorded best result of 87.43 and 84.85 per cent reduction in larval population of *Helicoverpa armigera*. Application of *B. thuringiensis* var. *kurstaki* in combination with neem oil, karanj oil, cotton seed oil and sesame oil at 5 per cent concentrations resulted in significantly higher larval mortalities in the range of 80 to 55 per cent.

Interaction effect of *B. thuringiensis* and different solvent extracts of *V. negundo* are presented in Table 39. Results clearly revealed that all treatment combinations except with acetone extract had resulted in supplemental synergism.

Lethal time required to kill 50 per cent population of *S. litura* larvae were determined in different treatments. From the Table 38, it is clear that *B. thuringiensis* when treated alone took 7.79 days for causing 50 per cent mortality to the test population, while it was greatly reduced upto 4.60 days in the treatment combination with methanol extract. By reducing the LT_{50} feeding period of the larvae can be decreased and thus the economic damage can be reduced to a greater extend. Combination treatment of *B. thuringiensis* with hexane extract also reduced the LT_{50} with a mean LT_{50} of 5.59 days (with 53.85% reduction in LT_{50}) . Water and acetone extracts of *V. negundo* in combination with *B. thuringiensis*

Fig. 46

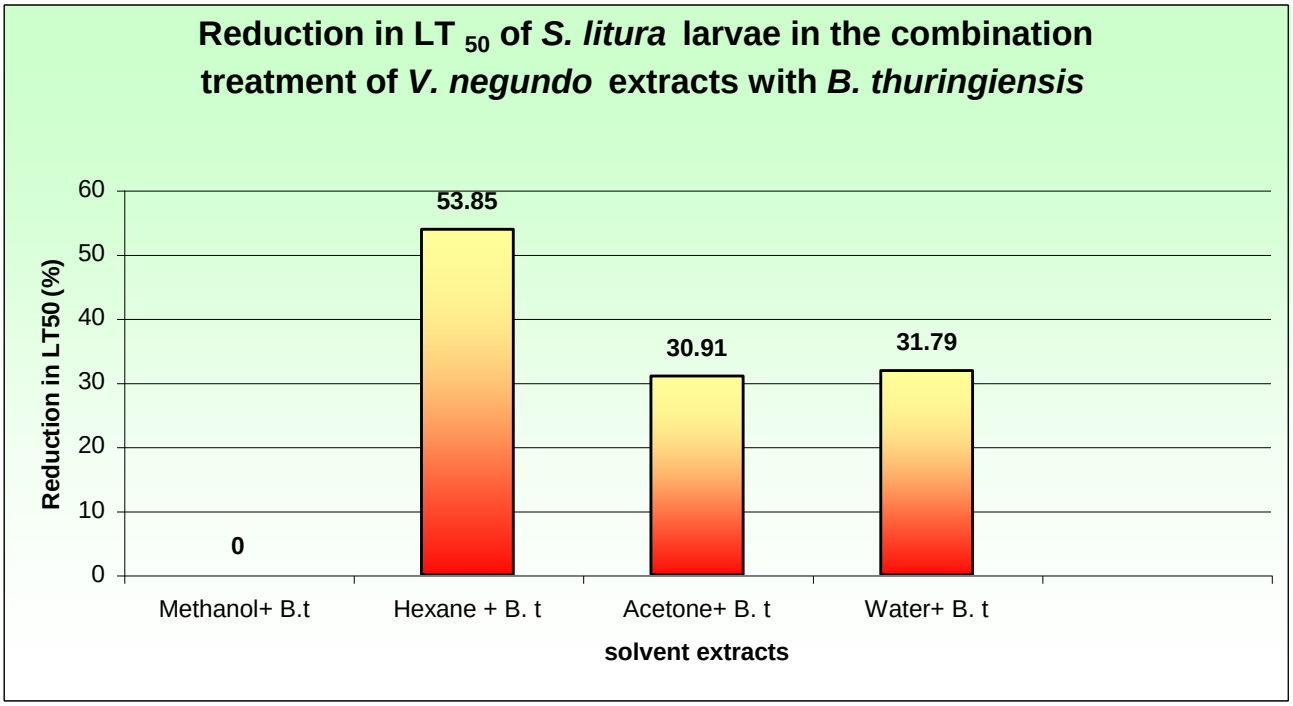
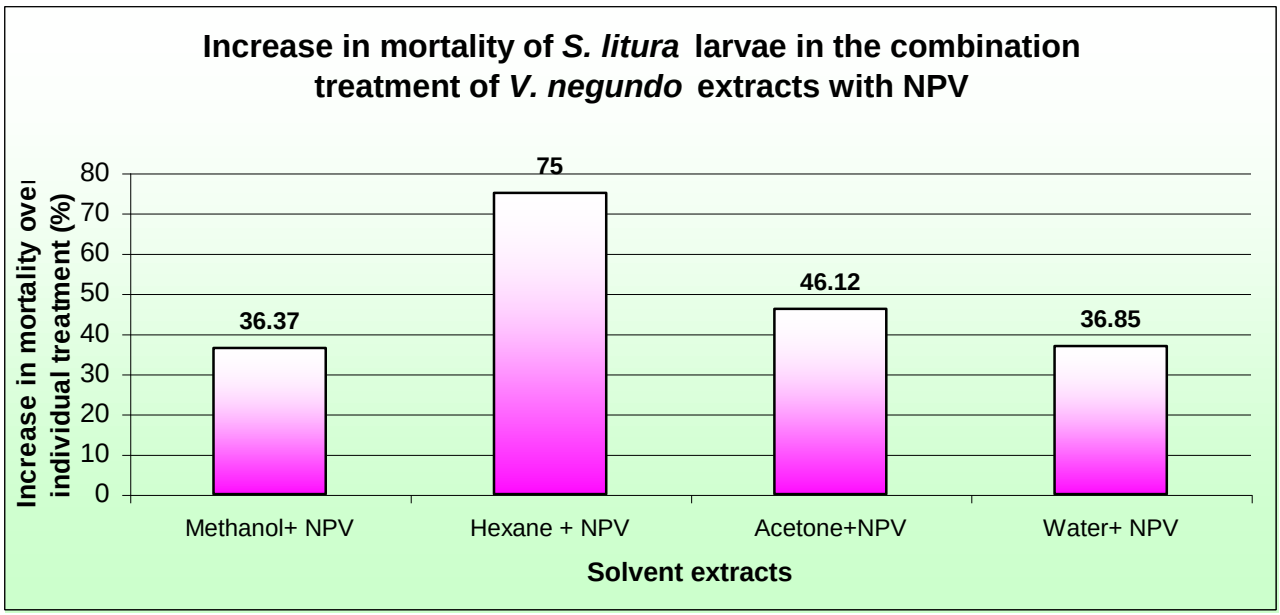

Reduction in LT 50 of S. litura larvae in the combination treatment of V. negundo extracts with B. thuringiensis
Reduction in LT50 (%)
60
50
40
30
20
10
0
53.85
30.91
31.79
0
Methanol+ B.t
Hexane + B. t
Acetone+ B. t
Water+ B. t
solvent extracts

Fig. 47


Increase in mortality of S. litura larvae in the combination treatment of V. negundo extracts with NPV
Increase in mortality over individual treatment (%)
80
70
60
50
40
30
20
10
0
36.37
75
46.12
36.85
Methanol+ NPV
Hexane + NPV
Acetone+NPV
Water+ NPV
Solvent extracts

also recorded lower LT_{50} values of 7.06 and 7.77 days respectively (with 31.79 and 30.91% reduction in LT_{50}) (Fig.46). Same solvent extracts when treated alone resulted in mean LT_{50} values of 11.29 and 10.63 days respectively. From this experiment it could be concluded that all the combination treatments exhibited temporal synergism in which lethal time required to kill 50 per cent population of the test insects is reduced in treatment combinations than in individual treatments. Similar were the findings of Hellpap (1984) in which combination treatment of neem seed kernel with *B. thuringiensis* increased mortality of *Spodoptera frugiperda* larvae and considerably reduced the LT_{50} and LT_{100}. Combination of sublethal concentrations of *B. thuriengiensis* spray formulation with azadirachtin not only enhanced the toxicity but also reduced the duration of action when used in a mixture. *B.t* – azadirachtin combinations of LC 50 + EC 50 results in 100 per cent mortality when treated against *H. armigera* (Singh *et al*., 2007).

5.5.2.4. Combined action of different solvent extracts of V. negundo and Nuclear polyhedrosis virus (NPV) against S. litura

An experiment was laid out in order to find out the efficacy of NPV in increasing mortality when combined with different solvent extracts of *V. negundo*. Results are presented in Tables 40 and 41 and Figs. 47 and 48.

Treatment with NPV alone resulted in a mean mortality of 60 per cent after 10 days of treatment. But combinations of *S. litura* NPV (Splt NPV) with *V. negundo* extract recorded very high mortalities ranging from 63.33 to 100 per cent. Combination treatment of methanol extract + NPV resulted in cent per cent mortality to *S. litura* showing 66.70 per cent increase in mortality than individual application of NPV to *S. litura*. This is in consonance with the finding of Devaprasad (1989) where in an additive effect was reported when NPV was combined with methanolic extract *Ocimum sanctum* and *A. calamus* against *H. armigera* and *S. litura*. Treatment combination of water extract with NPV also resulted in higher mortality of 86.67 per cent. Similar results were obtained by Rabindra and Jayaraj (1992) where in it was found that aqueous leaf extracts of *V. negundo* (10%) when applied along with Ha NPV @ $1.5 * 10^{12}$ POB / ha gave

better control of *H. armigera* and increased the grain yield. Hexane extracts recorded only 40 per cent mortality when applied alone, but in combination with NPV resulted in 75 per cent increase in mortality. The same trend was observed in acetone and water extracts in which combination treatments with NPV resulted in enhanced mortalities (46.12 and 36.85% in increase) (Fig.47).

The treatment with NPV alone took 8.18 days to kill 50 per cent of *S. litura* larvae, while in combination treatment with methanol extract LT_{50} was reduced to 2.58 days (3.17 times reduction in LT_{50} when compared to NPV alone). Hexane extract recorded highest LT_{50} of 12.09 days, but in combination with NPV, LT_{50} was reduced to 1.61 times when compared to treatment with hexane extract alone. Aqueous extract when applied alone recorded higher LT_{50} of 9.15 days, but in the combined application with NPV, LT_{50} was reduced to 1.35 times when compared to the individual application of aqueous extract. Methanol extract when applied alone took 4.65 days to cause 50 per cent mortality to *S. litura* while in combination treatment with NPV, LT_{50} was reduced to 2.58 days (44.52% reduction in LT_{50}). Hexane, acetone and water extracts in combination with NPV reduced LT_{50} considerably (37.8, 27.8 and 26.12% reduction in LT_{50}) (Fig.48). Experiments conducted by Sarode *et al*. (1997) recorded that the treatment combinations, NPV+ neem bitter and NPV + crude sugar recorded shortest LT_{50}. When combined with Spodavax (Splt NPV), the azadirachtin increased the per centage of larval mortality by 40-90 percentage when compared with treatments containing only the virus (Cook *et al*., 1996). Lethal time to cause 50 per cent mortality was reduced in the combination treatment with AZA + Splt NPV and was reported by Shapiro *et al*. (1994) and Cook *et al*. (1996). Combined effect of azadirachtin (AZA) and nucleopolyhedrosis virus of *S. litura* (Splt NPV) was evaluated by Nathan and Kalaivani (2006). They reported that combination treatment of AZA with Splt NPV(0.5 ppm + 1x 10^{6} OB) resulted in significantly higher larval mortality than treatment with either virus or AZA alone at the corresponding concentrations.

V. negundo extracts potentiates the effect of Splt NPV by producing mortality within a shorter time and negatively influences larval growth and time.

Fig. 48

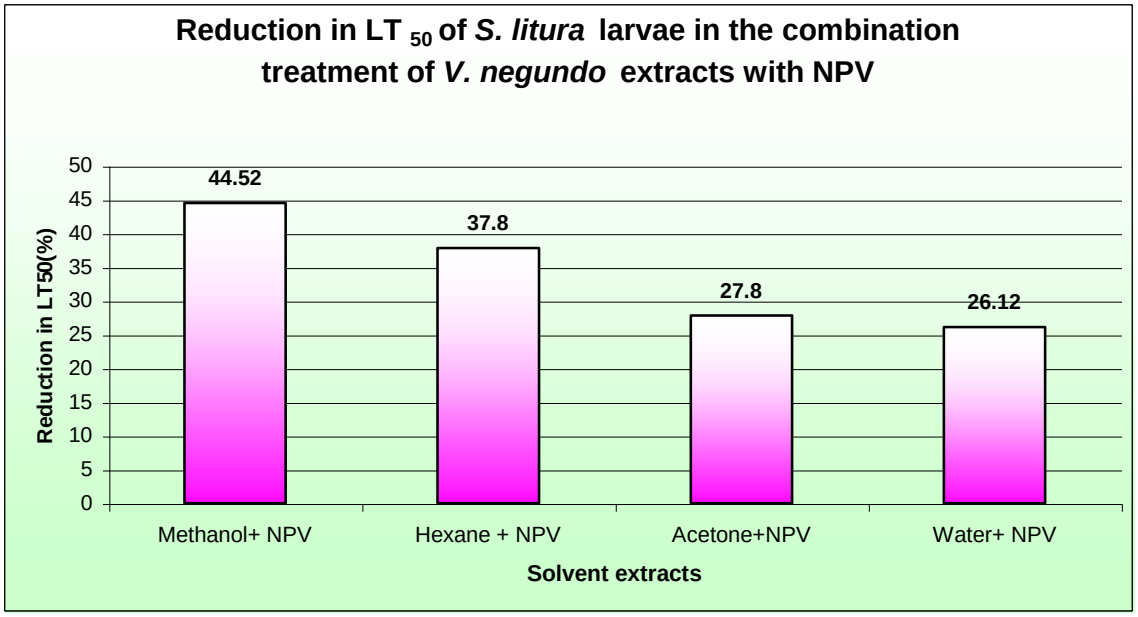
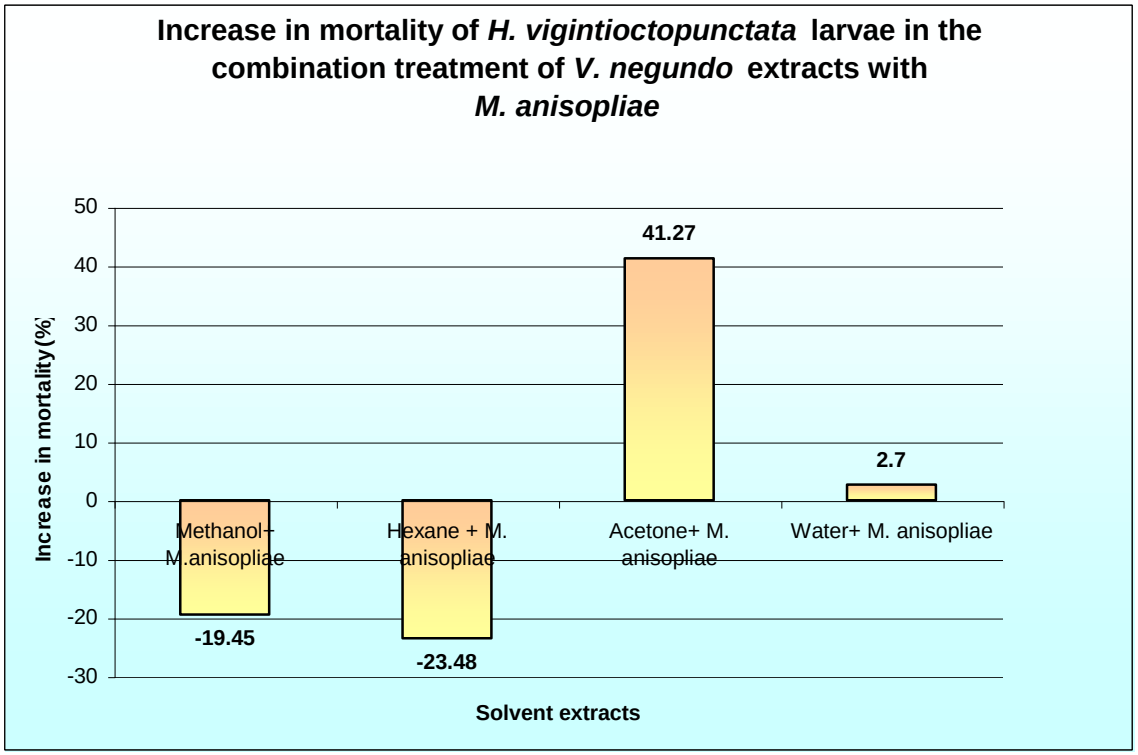

Reduction in LT 50 of S. litura larvae in the combination treatment of V. negundo extracts with NPV
Reduction in LT50(%)
50
45
40
35
30
25
20
15
10
5
0
44.52
37.8
27.8
26.12
Methanol+ NPV
Hexane + NPV
Acetone+NPV
Water+ NPV
Solvent extracts

Fig. 49


Increase in mortality of H. vigintioctopunctata larvae in the combination treatment of V. negundo extracts with M. anisopliae
Increase in mortality(%)
50
40
30
20
10
0
-10
-20
-30
41.27
2.7
-19.45
-23.48
Methanol+ M.anisopliae
Hexane + M. anisopliae
Acetone+ M. anisopliae
Water+ M. anisopliae
Solvent extracts

Solvent extracts of *V. negundo* kills a proportion of the larval population and the remaining survivors were then infected and killed by NPV also. Here *V. negundo* extracts weakens larval resistance to NPV. Azadirachtin treatment might have directly affected the gut lining in insects to allow easier pathogen penetration. (Cook *et al*., 1996). Exposure to a stressor might influence the susceptibility of the host to an active pathogen (Steinhaus, 1959). Biologically active allelochemicals from the plant products will penetrate the gut wall which allows easy penetration of the pathogens into the haemocoel (Cook *et al*., 1996).

The greatest advantage is faster kill and lesser feeding damage to the host plants. There will be a potential decrease in the pathogen dosage required to kill larvae. The present finding results in the synergestic interaction of *V. negundo* extracts with Splt NPV and this can be included as a successful component in the integrated management programmes (IPM) against *S. litura*.

5.5.2.5. Combined action of different solvent extracts of V. negundo and M. anisopliae against H. vigintioctopunctata

Joint action of microbials and *V. negundo* extracts were also conducted against *H. vigintioctopunctata* and the results are presented in Tables 42 to 45 and Figs.49 to 52.

From the Table 42, it is clear that upto 8th day after treatment, lowest mortality of 36.67 per cent was recorded in the treatment with *M. anisopliae* alone. All the combination treatments of *M. anisopliae* recorded higher mortalities of 63.3 (*M. anisopliae* + hexane), 53.33(*M. anisopliae* + acetone), 56.67 (*M. anisopliae* + water) and 50.00 (*M. anisopliae* + methanol).

After ten days of observations, treatment with hexane alone recorded highest mortality of 83.33 per cent and was followed by *M. anisopliae* alone treatment. Methanol extract alone recorded a mean mortality of 63.33 per cent and was on par with the treatment combination of *M. anisopliae* with hexane and acetone with the same mean per cent mortality. The combination treatments of acetone and water extracts with *M. anisopliae* resulted in enhanced mortalities

Fig. 50

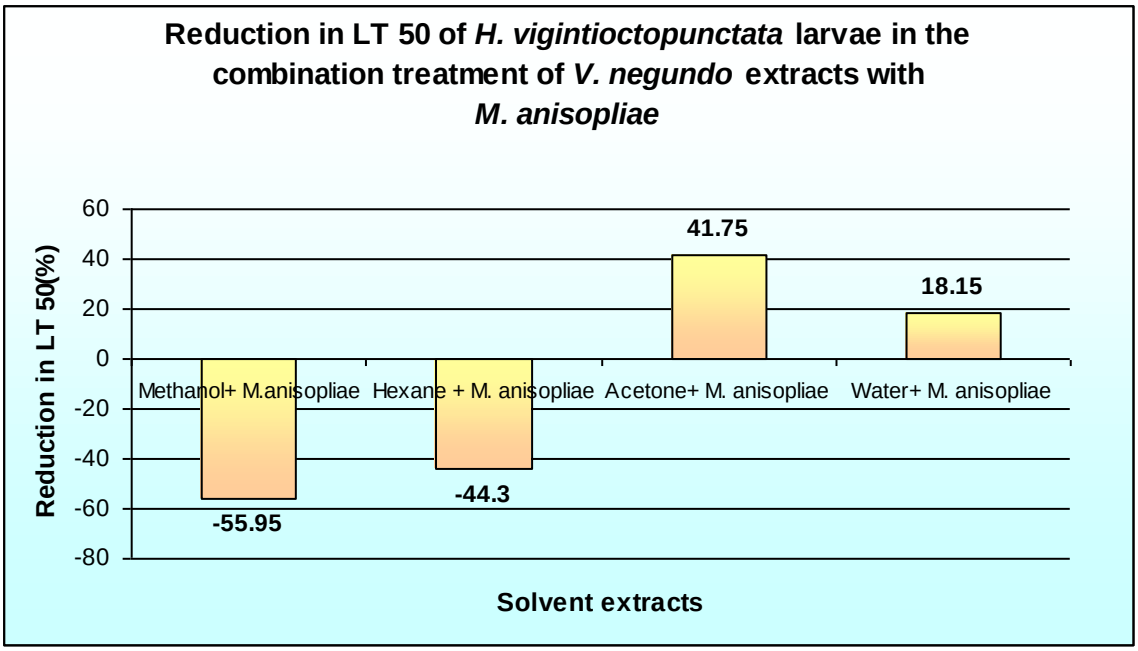
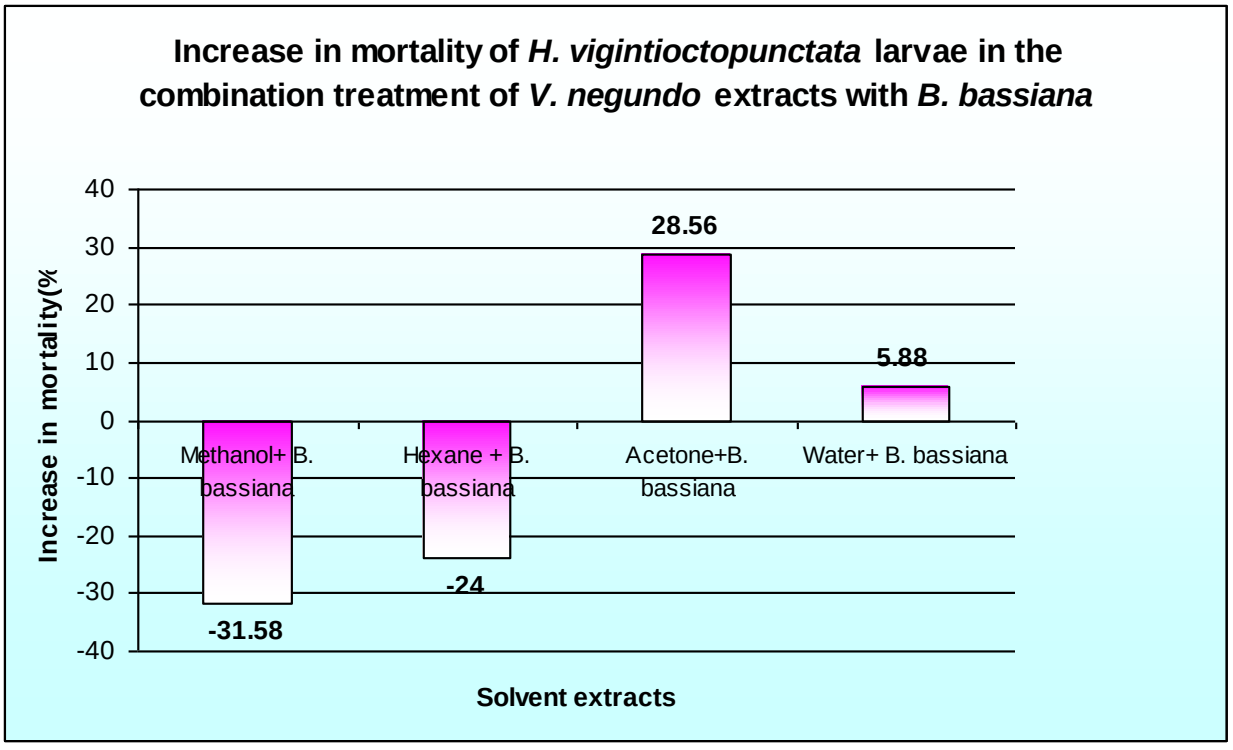

Reduction in LT 50 of H. vigintioctopunctata larvae in the combination treatment of V. negundo extracts with M. anisopliae
Reduction in LT 50(%)
60
40
20
0
-20
-40
-60
-80
41.75
18.15
-55.95
-44.3
Methanol+ M.anisopliae
Hexane + M. anisopliae
Acetone+ M. anisopliae
Water+ M. anisopliae
Solvent extracts

Fig. 51


Increase in mortality of H. vigintioctopunctata larvae in the combination treatment of V. negundo extracts with B. bassiana
Increase in mortality(%
40
30
20
10
0
-10
-20
-30
-40
28.56
5.88
-31.58
-24
Methanol+ B. bassiana
Hexane + B. bassiana
Acetone+B. bassiana
Water+ B. bassiana
Solvent extracts

(41.27 and 2.7% increase) when compared to the treatments in which they are applied alone (Fig.49).

Time mortality response studies revealed that treatment with *M. anisopliae* alone recorded longer time period of 8.2 days. All the combination treatments except *M. anisopliae* with methanol recorded lower LT_{50} values of 6.32 (*M. anisopliae* + hexane), 7.72 (*M. anisopliae* + acetone) and 7.44 days (*M. anisopliae* + water) respectively. Lethal time to kill 50 per cent population of *S. litura* was reduced considerably in combination treatments of acetone and water extracts with *M. anisopliae*. Acetone and water extracts when applied alone recorded LT_{50} of 10.85 and 9.09 days, while in combination treatments, LT_{50} was reduced to 6.32 and 7.44 days (with 41.75 and 18.15% reduction) respectively (Table 42 and Fig.50).

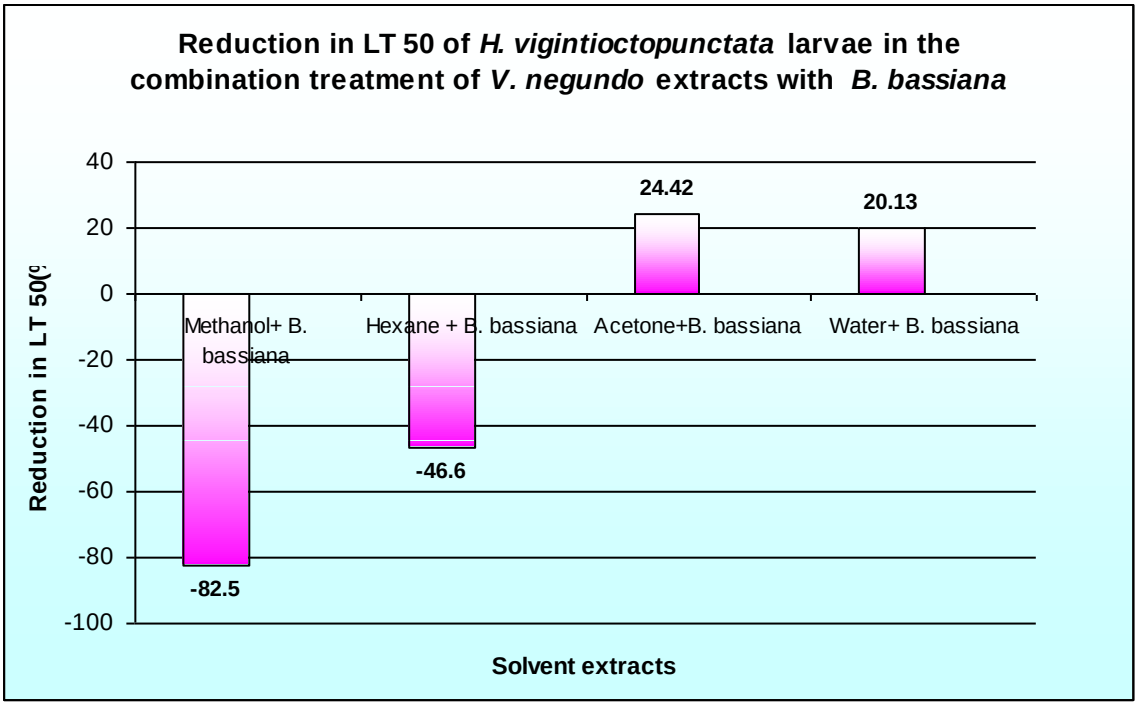
Interaction studies revealed that synergism was recorded in treatment combinations of *M. anisopliae* with hexane and acetone with the interaction effect being subadditive synergism. These treatments recorded synergestic coefficients of –0.007,-0.010 indicating submultiplicative synergism (Table 43).

5.5.2.6. Combined action of different solvent extracts of V. negundo and B. bassiana against H. vigintioctopunctata

Tables 44 and 45 and Figs.51 and 52 depicts results of the study. In treatment with *B. bassiana* alone, initial mortality was observed only from the third day onwards. In all other treatments except treatment combination of *B. bassiana* with methanol and acetone , mortality started from the first day onwards. Upto 8^{th} day after treatment, treatment with *B. bassiana* alone recorded very low mortality of 43.33 per cent while all other combination treatments except *B. bassiana* + methanol recorded higher mortalities of 63.33 (*B. bassiana* + hexane), 56.67 (*B. bassiana* + acetone) and 56.67 (*B. bassiana* + water) respectively (Table 44).

After ten days of observation, highest mortality was observed in treatment with hexane alone with 83.30 per cent mortality, it was then followed by *B. bassiana* alone treatment with 76.67 per cent mortality. Acetone and water

Fig. 52


Reduction in LT 50 of H. vigintioctopunctata larvae in the combination treatment of V. negundo extracts with B. bassiana
40
20
0
-20
-40
-60
-80
-100
24.42
20.13
-46.6
-82.5
Methanol+ B. bassiana
Hexane + B. bassiana
Acetone+B. bassiana
Water+ B. bassiana
Reduction in LT 50
Solvent extracts

extracts alone recorded low mortalities of 46.67 and 56.67 per cent respectively, but in combination treatments recorded higher mortalities of 60 each (with 28.56 and 5.88% increase) (Fig.51).

In case of *B. bassiana* alone treatment, LT_{50} value obtained was 8.89 days. All the treatment combinations except with methanol recorded lower LT_{50} values of 6.42(*B. bassiana* + hexane), 8.20 (*B. bassiana* + acetone) and 7.26 days (*B. bassiana* + water) respectively indicating temporal synergism (Table 44).

Methanol and hexane extracts in combination with *B. bassiana* recorded lower mortalities (31.58 and 24% reduction), when compared to the treatments in which they are applied alone. Time mortality studies revealed that the treatment combinations of methanol and hexane extracts with *M. anisopliae* resulted in increased LT_{50} (82.50 and 46.60% increase), when compared to their individual application and hence these combinations can be considered inferior. Combination treatments of acetone and water extracts with *B. bassiana* resulted in increased mortalities (28.56 and 5.88%) and reduced LT_{50} (24.42 and 20.13% reduction) when compared to the treatments in which they are applied alone (Fig.51 and 52).

Interaction effects presented in Table 45, revealed that the treatment combinations of *B. bassiana* with hexane, acetone and water resulted in subadditive synergism with synergestic coefficients of –0.0072, -0.013 and –0.012 indicating submultiplicative synergism. An experiment was done by Mohan *et al*., 2009 to test the influence of neem formulations on growth of *B. bassiana* and to evaluate their compatibility and synergism. About 30 isolates of *B. bassiana* were screened for their compatibility with neem and the results shown that the combined treatment was found to have an antogonistic effect on *S. litura* mortality when *B. bassiana* isolate sensitive to neem was used.

Summary

6. SUMMARY

The research work entitled " Biopotency of Indian Privet, *Vitex negundo* Linn. (Verbenaceae) against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae)" was undertaken in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur during 2005-2008.

The salient findings of the experiment covering bioefficacy of plant parts and solvent extracts of *V. negundo*, mode of action of *V. negundo* solvent extracts as with ovipositional deterrency, ovicidal action, growth and developmental effects, antifeedant action, nutritional effects, juvenomimetic activity, insecticidal and toxic effects, synergestic or antogonistic action in combination with microbials are summarized here under.

- Among the different parts *viz.,* leaves, shoots and flowers of *V. negundo,* leaves showed significant biological effectiveness against *S. litura* and *H. vigintioctopunctata*.
- *V. negundo* leaf extracts with all the solvents *viz.,* hexane, acetone, water and methanol at six per cent concentration caused more than 70 per cent deterrency for oviposition by *S. litura*. Methanol extract caused highest ovipositional deterrency (94.2%). Against *H. vigintioctopunctata* methanol extract resulted in cent per cent inhibition of egg laying.
- Methanol extract of leaves of *V. negundo* caused maximum mortality against *S. litura*, while against *H. vigintioctopunctata* hexane extracts resulted in maximum mortality.
- Maximum ovicidal action to *S. litura* was reported by methanol extract (6%) with 61 per cent inhibition of hatching.
- Against *H. vigintioctopunctata* both acetone and water extracts at four and six per cent concentrations resulted in cent per cent inhibition of hatching. Hexane extract at six per cent also resulted in significant ovicidal action with 93.6 per cent inhibition of hatching.

- Growth and developmental studies revealed that acetone extract at six per cent caused maximum reduction in pupal weight (55.25%) in *S. litura* larvae reared on castor, while in case of diet fed larvae, methanol and acetone extracts caused highest reduction in pupal weights (72.60 and 70% respectively). Banana reared *S. litura* larvae showed maximum reduction in pupal weight (65.52%) in treatment with methanol six per cent concentration.
- Significant reduction in pupation was observed in *S. litura* larvae treated with *V. neugndo* extracts. All the solvent extracts at six per cent caused more than 50 per cent reduction in pupation in case of castor and banana reared *S. litura* larvae. Hexane, acetone and methanol extracts resulted in more than 90 per cent reduction in pupation in castor reared *S. litura*, while in banana reared larvae, maximum reduction in pupation was observed in methanol extract at six per cent concentration (85.72%). The same trend was observed in diet fed larvae with 77.80 per cent reduction in pupation.
- Prolonged larval duration was observed in all treatments with *V. negundo* extracts and greater duration (19 to 26.33 days) was observed in castor and diet fed *S. litura* larvae, when compared to banana reared larvae. Castor fed larvae recorded maximum larval duration (19 days) on treatment with acetone extract (6%), in case of diet fed larvae, methanol extract (6%) resulted in maximum delay in pupation (26.33 days). None of the solvent extracts resulted in more than 50 per cent increase in larval duration in case of banana reared *S. litura* larvae.
- None of the solvent extracts resulted in more than 50 per cent leaf protection. Highest leaf protection (22.619 and 25.563 % respectively) was recorded by aqueous extract in both castor and diet fed larvae. In case of banana reared *S. litura* larvae both acetone (6%) and water extracts (6%) recorded higher leaf protection (42.39 and 41.12%) respectively.
- Antifeedat action was not recorded by *V. negundo* solvent extracts, when tested against *H. vigintioctopunctata*. Only acetone extract (6%) recorded higher leaf protection of 22.96 per cent
- Among the different solvent extracts of *V. negundo*, acetone extract (6%) resulted in maximum larval starvation (51.482%) in castor reared *S. litura* larvae, while in

case of diet fed larvae, methanol extract (6%) recorded highest larval starvation (44.239%). Both aqueous and methanol extracts (6%) recorded highest larval starvation (72.95 and 74.29 % respectively) in banana reared *S. litura* larvae.

- Against *H. vigintioctopunctata*, both hexane and methanol extracts recorded highest larval starvation (117.09 and 101.70%).
- All the castor and diet fed larvae resulted in increased AD when compared to control. Acetone (6%) recorded highest AD in both castor and diet fed larvae (96.12 and 96.56%), while banana reared *S. litura* larvae showed a reduction in AD up to 67.69 per cent on treatment with hexane extract six per cent.
- Hexane, acetone and methanol extracts of *V. negundo* (6%) were potential disruptors of digestion and assimilation in insect systems leading to very weak individuals of *S. litura* larvae with very low growth rate as proved by their lower ECI and ECD values. All these extracts resulted in more than 75 per cent reduction in ECI and ECD in case of *S. litura* larvae irrespective of the host food material on which it is reared.
- All the solvent extracts (hexane, acetone, water and methanol) at six per cent level resulted in more than 90 per cent reduction in both ECI and ECD in case of *H. vigintioctopunctata* proving their efficacy as potent growth disruptors.
- Acetone extract of *V. negundo* at six per cent level significantly reduced RCR in both castor and banana reared *S. litura* larvae (65.06 and 61.33% reduction respectively), whereas in diet fed larvae RCR was reduced only up to 29.20 per cent on treatment with acetone extract (6%). But, water and methanol extracts (6%) resulted in increase in RCR for both diet and banana fed *S. litura* larvae.
- In case of *H. vigintioctopunctata*, all the solvent extracts (hexane, acetone, water and methanol) at six per cent level resulted in an increase of RCR when compared to control.
- All the solvent extracts (hexane, acetone, water and methanol) at six per cent level caused significant reduction in the growth of *S. litura*. Maximum growth reduction was recorded by acetone extract at six per cent level (108.11%) followed by hexane and methanol extracts (97.22 and 97.44 %) in castor reared larvae of *S. litura*. Diet fed larvae exhibited complete growth inhibition on

treatment with methanol extract at six per cent level while in case of larvae reared on banana, acetone extract at six per cent recorded maximum growth reduction (92.86%).

- When applied against *H. vigintioctopunctata*, all the solvent extracts except aqueous extract (6%) acted as growth inhibitors with more than 100 per cent reduction in growth. Aqueous extract (6%) also reduced growth of grubs up to 86.21 per cent.
- Relative growth rate (RGR) showed a reducing trend with increase in concentrations of *V. negundo*. Maximum growth reduction in castor reared *S. litura* larvae were observed on treatment with acetone and methanol extracts at six per cent concentration (99.53 and 133.94 % respectively).
- In case of *H. vigintioctopunctata*, all the solvent extracts of *V. negundo* (hexane, acetone, water and methanol) at six per cent level resulted in more than 100 per cent inhibition of growth. Here, loss of weight has resulted instead of weight gain.
- Among the different solvent extracts, hexane and methanol extracts at (6%) induced maximum morphogenic malformations in *S. litura* larvae reared on castor, diet and banana.
- Cent per cent reduction in fecundity of *S. litura* was recorded by solvent extracts of hexane, acetone and water (6%). Methanol extract also reduced fecundity up to 98.81 per cent.
- In case of *H. vigintioctopunctata*, solvent extracts of hexane and water at six per cent resulted in cent per cent reduction of fecundity. Acetone and methanol extracts also reduced fecundity to a considerable level (91.02 and 91.18 % respectively).
- Treatments with *V. negundo* extracts (hexane, acetone, water and methanol) caused more than 80 per cent reduction in longevity of adults of *S. litura*
- Longevity was reduced to more than 90 per cent by solvent extracts of hexane and water at six per cent concentration when applied to *H. vigintioctopunctata*.
- Methanol extract of *V. negundo* (6%) recorded highest insecticidal action against *S. litura* larvae, while against *H. vigintioctopunctata*, hexane extract (6%) resulted in maximum mortality proving its potent insecticidal action.

- Toxicological studies basd on topical application in terms of LD_{50} values revealed that methanol extract (6%) was the most toxic solvent extract with lowest LD_{50} against *S. litura* while, against *H. vigintioctopunctata* hexane extract (6%) was the most toxic solvent extract.
- Compatibility studies revealed that all the *V. negundo* extracts except methanol extract were compatible to the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Highest inhibition was recorded by azadirachtin and quinalphos.
- *Bacillus thuringiensis* was found to be compatible with all the solvent extracts of *V. negundo*.
- Hexane, acetone and methanol extracts of *V. negundo* in combination with *B. thuringiensis* resulted in significantly higher mortalities (136.4, 58.06 and 14.29 % more than the individual application of hexane, acetone and methanol extracts) and very low LT_{50} values (53.85, 30.91 and 31.79% reduction in LT_{50}) when compared with the individual application of solvent extracts.
- Joint application of hexane extract of *V. negundo* with NPV on *S. litura* resulted in 75 per cent increase in mortality and 37.8 per cent reduction in LT_{50} when compared to the individual application of hexane extract to *S. litura*.
- Combined application of methanol extract of *V. negundo* with NPV on *S. litura* resulted in 36.37 per cent increase in mortality and 44.52 per cent reduction in LT_{50} when compared to the individual application of methanol extract to *S. litura*.
- Combination treatment of water extract of *V. negundo* with NPV also resulted in 36.85 per cent increase in mortality and 26.12 per cent reduction in LT_{50} when compared to the individual application of water extract to *S. litura*.
- Acetone extract when used in combination with NPV, mortality was enhanced to 46.12 per cent and LT_{50} was reduced to 8.15 days with 27.8 per cent reduction in LT_{50}.

Future line of work

- Field studies are to be conducted in order to test the efficacy of *V. negundo* extracts at field level.
- Phytochemical screening of leaf extracts of *V. negundo* can be done.

- Studies are to be conducted at the cellular and molecular levels to know whether there is disruption in gut epithelial cells/inhibition in the activity of digestive enzymes.
- Protein and enzyme studies are to be conducted to know the level of defence enzymes and if there is inhibition in the detoxification enzyme levels by the *V. negundo* extracts, it can be well used in insect resistance management programmes.
- *V. negundo* extracts act as IGR compounds, works are to be conducted to know whether it is due to inhibition of ecdysone monooxygenase enzyme or due to the inhibition of the receptor protein.

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*Originals not seen

BIOPOTENCY OF INDIAN PRIVET, *Vitex negundo* Linn. (Verbenaceae) AGAINST *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* Fab. (Coleoptera: Coccinellidae)

By

DEEPTHY. K.B

THESIS

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Department of Agricultural Entomology

COLLEGE OF HORTICULTURE

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ABSTRACT

The present investigations on "Biopotency of Indian privet, *Vitex negundo* Linn. (Verbenaceae) against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) and *Henosepilachna vigintioctopunctata* (Coleoptera: Noctuidae) were carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara during 2005-2008. The objectives of this study were to screen the different parts (leaf, shoot and flower) of *V. negundo* for its biological efficiency with different solvent extracts against *S. litura* and *H. vigintioctopunctata* and to test the *V. negundo* extracts for their biological responses as with ovipositional deterrency, ovicidal action, antifeedancy, morphogenic effects and reproductive inhibition against the test insects. Experiments were also conducted to assess the potency of *V. negundo* extracts in combination with different entomopathogens.

Screening experiments revealed that among the different parts *viz*., leaves, shoots and flowers of *V. negundo*, leaves showed significant bio response against *S. litura* and *H. vigintioctopunctata*. Methanol and hexane extract of leaves of V. *negundo* at six per cent resulted in maximum mortality of *S. litura* and *H. vigintioctopunctata.*

V. negundo extracts with methanol (6%) indicated significant ovipositional deterrency with 94.02 and 100 per cent reduction in egg laying of *S. litura* and *H. vigintioctopunctata* respectively.

Methanol extract (6%) proved as an efficient ovicidal agent against *S. litura*. Against *H. vigintioctopunctata* acetone aqueous extracts showed pronounced ovicidal action at lower concentration of four per cent resulting in cent percent reduction in hatching.

Studies on growth and developmental effects of *V. negundo* extracts revealed that methanol and acetone extracts resulted in maximum reduction in pupal weight and pupation of *S. litura*. Delay in moulting of *S. litura* was observed in different treatments with *V. negundo*. *S. litura* reared in treated castor leaves and semi synthetic diet recorded maximum larval duration (19 and 26 days respectively) with acetone (6%) while water extract resulted in greater duration of 17.67 days in banana fed larvae.

V. negundo cause no antifeedant action against *S. litura* and *H. vigintioctopunctata*. Food consumption and utilization studies on *S. litura* and *H. vigintioctopunctata* revealed that *V. negundo* extracts caused a drastic decline in growth parameters like, Efficiency of Conversion of Ingested Food (ECI) and Efficiency of Conversion of Digested Food (ECD), larval growth and Relative Growth Rate (RGR) thus indicating the inhibitory action of *V. negundo* on the growth of test insects. All the solvent extracts (except aqueous extract) reduced ECI and ECD against both *S. litura* and *H. vigintioctopunctata* proving the potency of *V. negundo* as an efficient growth inhibitor. Acetone extract (6%) resulted in maximum reduction in RCR of *S. litura* and *H. vigintioctopunctata*. Hexane, acetone and methanol extracts caused highest growth inhibition in *H. vigintioctopunctata*. Correlation studies revealed that there is a highly significant positive correlation of ECI and ECD with RGR both in *S. litura* and *H. vigintioctopunctata*.

Solvent extracts of *V. negundo* were found to induce pupal and adult malformations in *S. litura* and *H. vigintioctopunctata*. Hexane and methanol extracts caused highest pupal and adult malformations in *S. litura* larvae. All the solvent extracts (6%) caused significant reduction in longevity and fecundity of both *S. litura* and *H. vigintioctopunctata*. Methanol extract was proved to be the most toxic (least LD_{50} value) against *S. litura* and against *H. vigintioctopunctata*, hexane extract showed maximum toxicity.

Compatibility studies revealed that methanol extract inhibited growth of both *Metarhizium anisopliae* and *Beauveria bassiana*. Combination treatment of methanol extract with *M. anisopliae* resulted in reduction in mortality of *S. litura* leading to antagonistic interaction. Combination studies conducted with *Bacillus thuringiensis* and Nuclear Polyhedrosis Virus (NPV) resulted in enhanced mortality and reduction in Median Lethal Time (LT_{50}).

The results of the present study thus indicate the multiple modes of action of *V. negundo* against insect pests and hence there is good scope of its utilization as an efficient component in Integrated Pest Management (IPM) programmes against *S. litura* and *H. vigintioctopunctata*.