OIL BASED BIOPESTICIDE FROM *Andrographis paniculata* (Burm.f.) Nees AGAINST SUCKING PESTS OF COWPEA

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by

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(2018-11-068)

THESIS

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2020

<u>DECLARATION</u>

I, hereby declare that this thesis entitled **"OIL BASED BIOPESTICIDE FROM *Andrographis paniculata* (Burm.f.) Nees AGAINST SUCKING PESTS OF COWPEA"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"OIL BASED BIOPESTICIDE FROM *Andrographis paniculata* (Burm.f.) Nees AGAINST SUCKING PESTS OF COWPEA" is** a record of research work done independently by Ms. Anuja Raveendran (2018-11-068) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Ms. Anuja Raveendran** (2018-11-068), a candidate for the degree of **Master of Science in Agriculture** with major in **Agricultural Entomology**, agree that the thesis entitled **"OIL BASED BIOPESTICIDE FROM *Andrographis paniculata* (Burm.f.) Nees AGAINST SUCKING PESTS OF COWPEA"** may be submitted by **Ms. Anuja Raveendran** (2018-11-068), in partial fulfilment of the requirement for the degree.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

et al.	And other co workers
a.i.	Active ingredient
CD	Critical difference
CRD	Completely Randomized Block Design
DAP	Days after planting
DAS	Days after spraying
DAT	Days after treatment
@	At the rate of
BPF	Biopesticide formulation
Cm	Centimetre
EC	Emulsifiable Concentrate
FYM	Farmyard manure
Fig.	Figure
G	Gram
Ha	Hectar
HAT	Hours after treatment
KAU	Kerala Agricultural University
kg ha-1	Kilogram per hectare
L	Litre
mL	Millilitre
viz.	Namely
NS	Non-significant
%	Per cent
PEA	Plant extract of *A. paniculata*
ha-1	Per hectare

Rpm	Revolutions per minute
sp. or spp	Species (Singular and Plural)
ie.	That is
WG	Wettable granules
T	Triton X-100
CIBRC	Central Insecticide Board and Registration Committee

Introduction

1. INTRODUCTION

Pulses are one among the key food crops cultivating globally due to its greater protein content. India is world's leading producer of pulses. Pulses form an integral part of Indian diet providing much needed protein to the carbohydrate rich diet. The country has an export potential of 2,70,811.16 MT of pulses to the world for the worth of Rs.1,679.98 crores/ 242.66 USD Millions during the year 2018-19 (APEDA, 2012).

Pulses accounts for 20 to 25 per cent protein by weight which is twice the protein content of wheat and three times that of rice. Major pulses comprise chickpeas (Bengal gram), pigeon pea (arhar or tur), urd beans (black matpe), moong beans, masur (lentil), peas, and beans of various types.

The cowpea *Vigna unguiculata* (L.) Walp.is an annual herbaceous legume cultivated for grain and vegetable purpose. Due to its improved tolerance for low rainfall and sandy soil, it is an imperative crop in the semi-arid regions across Africa and Asia. As the plant's root nodules are able to fix atmospheric nitrogen, it demands very few inputs making it a valued crop for resource deprived farmers. Cowpea is well-suited for intercropping with other crops and the entire plant is used as forage for animals, with its use as cattle feed likely responsible for its name (Quin, 1997).

Among the diverse constraints responsible for lower yield of cowpea, insect pests cause substantial losses. The avoidable losses in yield due to insect pest are estimated to be in the range of 66-100 per cent (Pandey *et al*., 1991). Insect pests of cowpea include aphids (*A. craccivora* Koch), leaf miner (*Liriomyza trifolii* Burgess), leafhopper (*Empoasca* sp.), whitefly (*Bemisia tabaci* Genn.), thrips (*Megalurothrips sjostedti* (Trybom)), green stink bug (*Chinavia hilaris* Say), blue butterfly (*Lampides boeticus* Linnaeus), lablab bug (*Coptosoma cribraria* (Fabricius)), *Ootheca* sp., *Clavigralla* sp., *Maruca* sp., *Etiella* sp., *Helicoverpa* sp. and tobacco leaf eating caterpillar (*Spodoptera litura* (Fabricius)). They generally cause low yield and sometimes leads to total yield loss and complete crop failure due to the activities of a spectrum of insect pests which ravage the crop in the field at different growth stages (Patel *et al*. 2012). Oyewale and Bamaiyi, (2013) reported that sucking pests, excrete copious amount of honeydew on the plant parts which interfere with the photo synthetic activity and ultimately reducing the yield of crops.

The introduction of high yielding varieties and intensive cultivation under umbrella of chemical pesticides have amplified the pest problem in recent decades. Growers often rely up on chemical pesticides for managing these pests on large scale and due to extreme and unselective use of these pesticides have resulted in several problems like development of resistance, elimination of natural enemies, resurgence of secondary pest, contamination of water, soil and food chain and ultimately results in environmental pollution and ecological instability .In cowpea harvesting is done at short intervals and hence it is not advisable to use insecticides having long residual action for pest control (Khade *et al*., 2014). Hence, researchers are focusing on an effective, environmentally safe, and biorational control measures to minimize these problems.

The natural plant products (botanicals) meet this criterion and have immense potential to influence modern agro-chemical research. The use of botanical pesticides is now evolving as one of the prime means to safeguard crops and their products and the environment from pesticide pollution. Botanicals degrade rapidly than most chemical pesticides, and are, therefore, considered relatively environment welcoming and less likely to execute beneficial pests than synthetic pesticides with longer environmental retention (Guleria and Tiku, 2009).

A. paniculata (Burm.f.) Nees (Acanthaceae) is a bitter annual herb privileged with an active ingredient of insecticidal value. Chemical analysis showed that it contained two diterpenes which is neo-androgropholide and andrographolide. In addition, a new flavone 2-glucoside was reported in *A. paniculata* and *A.alata*. In ancient herbals *Andrographis* spp. claim to possess medicinal properties as well and hence it was recommended for malaria, snake bite, digestive ailments and dysentery. Oils of plant origin namely castor, neem and pongamia are also receiving more attention in current scenario, since they also can be better alternatives to conventional insecticides and promising botanicals (Bright *et al*., 2001).

The individual botanicals are not able to control crop pests when the pest population is very high in the field conditions. In view of this, a need was felt to find a sound and reliable Biopesticide formulation (BPF) which could be applied when insect pressure is high under field conditions. In this context, a project entitled “Oil based biopesticide from *A. paniculata* (Burm.f.) Nees against sucking pest of cowpea” has been made with the objective to develop and evaluate oil based ready to use biopesticide from *A. paniculata* and to fix the optimum dose for the management of sucking pests of cowpea.

Review of literature

2. REVIEW OF LITERATURE

Cowpea (*V. unguiculata*) is often referred to as the poor man's meat as it is a significant source of protein, vitamins and minerals for the rural poor who have limited access to protein from animal sources such as meat and fish (Timko *et al*., 2007). In India, the major constraint for cowpea production is insect pest resulting in lower production and productivity due to direct or indirect damage. As many as 21 insect pests of different groups have been recorded damaging the cowpea crop from germination to maturity stage (Patel *et al*., 2010; Kumar *et al*., 2017). Cowpea is attacked by different species of insect pests, among them, sucking pests *viz.*, aphid, *A. craccivora*, flower thrips, *Megalurothrips sjostedti* Trybom, leafhopper, *Empoasca kerri* Pruthi etc. are of major importance (Swarupa *et al*., 2019).

The use of synthetic insecticides has been the most widely used control measure and their uses has led to numerous threats such as residue problems, environmental pollution, pest resurgence and secondary pest outbreak. This has necessitated the search for alternative control measures that are eco-friendly and pose no health and environmental threat. The use of plants in insect pest management is not only useful for suppression of pest population but also helps to maintain the sound ecological balance (Adesina and Enudeme, 2018).

The publications related to the sucking pests of cowpea and their management, particularly the environmental sound measures employing botanical insecticides are reviewed here.

2.1 SUCKING PESTS OF COWPEA

The important insect species attacking cowpea crop include aphid (*A. craccivora*), leafhopper (*E. kerri*), thrips (*Megaleurothrips* spp.), leaf miner (*Acrocercops caerulea* Meyrick), whitefly (*Bemisia tabaci*, Genn.), spotted pod borer (*Maruca vitrata* (Fab.) and tobacco leaf eating caterpillar (*Spodoptera litura* Fab.) (Chadha, 2001).

Pandey (1991) reported that losses in foliage or grain of cowpea ranges from 20 to 100 per cent due to insect pests.

2.1.1 *A. craccivora*

The cowpea or groundnut aphid, *A. craccivora* is considered to be an important field pest of cowpea in Africa, Asia and Latin America (Singh and Jackai, 1985).

Legumes, including a varied range of cultivated species, are the major hosts of *A. craccivora,* but the insect is not restricted to this family. As many as five biotypes of *A. craccivora* have been documented (Kranz *et al*., 1977). Host preference studies indicates that cowpea promotes highest growth and reproduction of the insect. A common pattern in the tropics is for *A. craccivora* to spend the dry or winter season on wild hosts and weedy species such as *Melilotus* spp., *Medicago* spp., *Trifolium* spp., *Euphorbia* spp., *Boerhaavia* spp., as well as on volunteer growth of legume crops. The aphid (alatae) disperses soon after the start of monsoon. (Highland and Roberts, 1984).

Ofuya (1997) reported that adults are mostly black or dark brown, variable in size, being from 1.5 to 2 mm long, siphunculi and cauda black; antennae are about two thirds as long as the body. Nymphs are wingless, dusty brown or dark and impartially rounded in body shape. *A. craccivora* was the profuse vector on cowpea both in the first and second growing seasons. Aphids were most abundant and virus disease-infection rate correspondingly quicker in the wetter first growing season than in the second one. Both non-colonizing and colonizing aphids are significant in the epidemiology of CAMV (Atiri, 1984).

The virus-vector-host relationships of cowpea aphid-borne mosaic virus (CAMV) and its vector, *A. craccivora*, were studied in cowpea lines differing in resistance to aphid infestation by Atiri *et al.* (1986) and found that CAMV was acquired and inoculated by its vector during brief probes, confirming that it is non-persistently transmitted.

2.1.2 Pod bugs

Pod sucking bugs are the most devastating pests during the reproductive phase of cowpea causing considerable economic loss by affecting quantity and quality of the produce (Kavitha, 2010)

Clavigralla gibbosa Spinola and *Clavigrella horrens* Dohrn were reported as important pests during post flowering phase of cowpea. (Lefroy, 1909). Cowpea, pigeon pea, Lab lab and Cluster bean were reported as most preferred hosts of *C. gibbosa* (Singh et al., 1988). This pest has been reported from Karnataka, Maharashtra, Delhi, Madhya Pradesh, Uttar Pradesh, Tamil Nadu and Orissa (Srivastava, 1996). In tropical Africa, *Clavigrella* *tomentosicollis* Stal. was reported as the most destructive pest of cowpea causing premature drying and shriveling of pods resulting in a yield loss of 80% or more if left un controlled (Ekesi, 1999).

Regular infestation by coreid bug *Cletus* sp. in cowpea was observed by Faieiro et al. (1986). In Uttar Pradesh population bloom of green stink bug *Nezara viridula* (Linnaeus) was reported which was in correlation with relative humidity as well as maximum and minimum temperatures (Singh et al., 2002)

Visalakshi et al. (1976) reported heavy incidence of *Riptortus pedestris* (Fab.) in cowpea fields of Kerala regions. Feeding punctures rendered older pods unfit for consumption and tender pods failed to develop completely. *Riptortus linearis* (Fab.) were observed as the most serious sucking pest of cowpea causing a yield loss up to 79 percentage (Prayoga and Suharsona, 2005)

2.2 NATURAL ENEMIES

As per reports of Niba (2011), Major arthropod natural enemy populations recorded at cowpea experimental plots from vegetative to maturity stages were ladybird beetles (Coccinellidae, 50%), spiders (Arachnida, 11%), Wasps (Vespidae, 28%) and Assassin bugs (Reduviidae, 18%).

Among the arthropod natural enemies recorded in cowpea fields, the coccinellid, *Cheilomenes lunata* (Fabricius) was reported as a major predator of aphids. (Brown, 1972). However, it is not solely efficient in protecting the susceptible varieties from damage (Aalbersberg *et al.*, 1988). Heinrichs and Barrion (2004) reported that assassin bug *Rhinocoris segmentarius* (Germar) as observed to feed on caterpillars, and nymphs of pod-sucking bugs. These bugs are generalist predators that are also found on legumes and rice throughout Africa.

Seasonal abundance of cowpea aphid and its natural enemies were studied by Jangu (2005). Results revealed that coccinellid predators including *Menochilus sexmaculata* Fabricius and *Coccinella septumpunctata* (Linnaeus) were positively correlated with aphid infestation whereas abiotic factors that is minimum and maximum temperature were inversely related to aphid and coccinellid population.

Gauns *et al.* (2014) stated that lady beetle population was active throughout the cropping period with initial occurrence noticed at 2^{nd} week of August in Rahuri city of

Ahmednagar. Minimum temperature and evening relative humidity were negatively correlated with lady bird population.

The findings of Hatano *et al*. (2008) revealed that undamaged host plants emit volatiles that attracts aphid parasitoids, but not predators. Semiochemicals that originate from aphids (honey dew, alarm pheromones or smell of aphid itself) will help in locating host by natural enemies. Host acceptance is aided by contact chemicals for parasitoids and predators. Host recognition in parasitoids are based on contact chemicals on aphid cuticle or visual cues whereas host acceptance is based on unknown substance in aphid haemolymph.

Choudhary *et al*. (2017) conducted field experiment to investigate the influence of abiotic and biotic factors on incidence of aphid and results revealed that population of *M. sexmaculatus* and *C. septumpunctata* were positively correlated (r = 0.82 and r = 0.72 respectively) with aphid population. Population of predator was found to increase with aphid population. There was no significant correlation with population of *M. sexmaculatus* and *C. septumpunctata* with abiotic factors like minimum and maximum temperature, RH and rainfall.

2.3 MANAGEMENT OF SUCKING PESTS

In the view of importance of cowpea as source of proteinaceous food, improvement in its yielding capacity is necessary. One of the major obstacles in improving yield potential is pest incidence. Farmers often go for a minimum of 12 to 15 rounds of the conventional insecticide sprays to manage the sucking pest of cowpea.

Insect pests are the most important constraint to cowpea production. At least 20 major pest species were reported in various cowpea producing regions of the world in which the number vary from region to region. Thamilarasi (2016) reported that sucking pests *viz*., pea aphid, *A. craccivora*, pod bug, *Riptortus pedestris* (Fabricius), mealy bug, *Ferrisia virgata* (Cockerell), thrips, *Ayyaria chaetophora* (Karny), spotted red mite, *Tetranychus truncatus* (Ehara), pod borer, *Lampides boeticus* (Linnaeus), tobacco caterpillar, *Spodoptera litura* (Fabricius), and American serpentine leaf miner, *L. trifolii* are the pests associated with cowpea crop.

2.3.1 Chemicals

Oyewale and Bamaye (2013) commented that, over the years, chemical pesticides had made a countless contribution to the fight against pests and diseases. However, their extensive and long-term use resulted in insecticide resistance and biomagnifications of insecticides, which in turn resulted in margins on their export. Problems like soil and water contamination and vivid increase of the harmful residues in many primary and derived agricultural products arose which threatened both the general environment and human health.

Shen and Zhang (2000) observed that the use of synthetic organic insecticides in crop pest control programs globally had caused tremendous damage to the environment, pest resistance to insecticides, pest resurgence and lethal effects on non-target organisms. Practically no yields were obtained under no insecticide protection. Complete crop loss may occur especially in situation where management strategies are not undertaken.

A study by Abd-Ella (2014) made a comparison between the LC50s of the tested neonicotinoid insecticides for the cowpea aphid, *A. craccivora*, under laboratory conditions and results showed that the most toxic insecticide by ppm was thiamethoxam followed by acetamiprid, imidacloprid and dinotefuran.

Anandmurthy *et al.* (2017) reported that application of dinotefuran 0.006% dimethoate 0.03% and acetamiprid 0.004% proved effective in recording least aphid population.

Rai *et al.* (2013) found that insecticides like profenophos 50EC as most efficient against leaf miner infestation on upper leaves accompanied by thiamethoxan 20 SG, imidachloprid 600 FS, azadirachtin (1 500 ppm), profenophos 40% + cypermethrin 4%, Dimethoate 50 EC, Cypermethrin 25 EC and Endosulfan 35 EC over control.

Al-Kazafy *et al.* (2015) tested toxicity of three modern insecticides (etofenprox, imidacloprid and spirotetramat) against adults of sweetpotato whitefly, *B. tabaci* and American serpentine leafminer, *L. trifolii* under greenhouse conditions. The results revealed that imidacloprid was more toxic to adults of sweetpotato whitefly and American serpentine leafminer followed by etofenprox and spirotetramat after three treatments. The efficacy against leafminer were 78.7, 57.1 and 18.4%, respectively. The leafmines reduction was 77, 54.5 and 11.7%, respectively.

The efficacy of ten insecticides and bioagents against sucking insect pests, *viz.*, leaf hopper, *E. motti*, whitefly, *B. tabaci* and aphid, *A. craccivora* of cluster bean *Cyamopsis tetragonoloba* (Linn.) was studied by Yadav *et al.* (2015) and concluded that dimethoate (0.03%), thiamethoxam (0.025%) and imidacloprid (0.005%) proved to be the most effective. The profenophos (0.05%), acephate (0.037%), and lambda-cyhalothrin (0.008%) were ranked in the middle order of effectiveness; whereas, novaluron (0.02%), diflubenzuron (0.05%), *Metarrhizium anisopliae* (Metchnikoff) (2 x10^7 spores l^{-1}) and NSKE (5.0%) were proved to be the least effective.

Another Study was conducted by Yadav *et al.* (2011) to determine the efficacy of various biological control agents and insecticides agents against sucking insect pests of cluster bean i.e. *E. motti*, *B. tabaci* and *A. craccivora* and found thiamethoxam (0.025%), dimethoate (0.03%), imidacloprid 0.005%) and acephate (0.037%) were the most effective treatments in controlling *A. craccivora*. The highest seed yield was also found in the plots treated with thiamethoxam, dimethoate and imidacloprid.

2.3.2 Botanicals

Weinzierl (2000) highlighted the history of use of conventional pesticides such as neem, rotenone, sabadilla and pyrethrum. The outcomes of investigation showed that botanical mixtures could form the basis for a successful formulation and commercialization of biopesticides in developing countries, where low input agriculture is in vogue. In Nigeria, these plants are readily available in markets all the year round. Since the materials are used in ethno-botany for the treatment of various ailments, they are cheap, safe, easily biodegradable, and technologically and environmentally friendly. They could become valuable substitutes to the synthetic insecticides in the management of post flowering insect pests of cowpea in limited resource farmers farms.

Plant based insecticides (PBI) have been used for many centuries among marginal farmers in developing countries to control insect pests of both field crops and stored produce, but their potential was limited and ignored initially. Nicotine, pyrethrum and rotenone were popular among the PBIs used to some extent for storage pest control and other pests in green houses (Schmutterer, 1981).

Some of these plant species retain one or more useful properties such as antifeedancy, repellency, fast knock down, flushing action, biodegradability, broad-spectrum of activity and ability to reduce insect resistance (Olaifa *et al*., 1987; Stoll, 1988).

However, most of them are either feeble as insecticides or may require other plant species with different mode of action (depending on the rate of application and ratio) to improve their potency (Sommers, 1983; Oparaeke, 2004).

Botanical insecticides are mostly approved for managing insect pests of vegetables and fruit crops considering the synthetic organic pesticides are outrageous for causing residue problems on the crop yield. Plants are endowed with an enormous possibility to produce a wide range of allelochemicals that protect them from insect-pests. On the other hand, the production of phytochemicals has been reported to vary from plant to plant (Ahmad and Aqil, 2007). The phytochemicals derived from plants in response to insect pests attack, affect the feeding and ovipositional activity of insects on plants (Ramya *et al*., 2008).

Plants are known to have a variety of secondary metabolites that are essential for their growth and development and are indispensable in protection against pathogens and predators (Rosenthal, 1991). Isman (2000) revealed that secondary metabolites of plants are proved to have insecticidal, antifeedant, growth-regulating and repellent properties.

There is resumed inquisitiveness in botanical pesticides and a number of the phytochemicals such as pyrethrin (Casida, 1980), plant essential oils (Koul *et al*., 2008; Schafer and Wink, 2009), azadirachtin (Khater, 2012), nicotine (El-Wakeil, 2013), ryanoids (Martina and Kristina, 2013) which have been developed as commercial botanical insecticides.

2.3.2.1 Bioefficacy of Andrographis paniculata

A. paniculata is a shrub referred as "Kalmegh" and was most commonly used as wonder drug in traditional medicine and ayurvedic and it is very famous for its clinical applications (Mishra *et al.,* 2007). *A. paniculata* contains many primary constituents, diterpenoid lactones (Andrographolides), paniculides, farnesols and flavonoids conferring insecticidal potential (Ramya *et al.*, 2011).

Singh *et al.* (2014) tested bioefficacy of different botanical pesticides i.e. *A. paniculata*, *Catharanthus roseus (*L.), *Calotropis gigantea* (L.), *Lantana camara* L., *Azadirachta indica* A. Juss., *Pongamia pinnata* L. and *Cassia tora* L. Among the botanical pesticides, decoction from *A. paniculata* was more effective against thrips (3.73–5.01 thrips per leaf) and the efficacy was similar to 0.03% dimethoate. The highest yield was obtained in dimethoate (98.07 q ha^{-1} and *A. paniculata* decoction spray (98.04 q ha^{-1}) as against the control plot (89.99 q ha^{-1}).

In pot culture experiment Least population of chilli aphids, mites and thrips were recorded at 5% concentration of extract of A. paniculata + pongamia oil + Triton X- 100 sprayed chilli plants and the effects were on par with chemical check thiamethoxam 25% WG and spiromesifen 22.9% SC (Bhavyasree, 2018)

Prema *et al.* (2018) detected that leaf extract of *A. paniculata* 10 % was found to be effective against *Thrips palmi* Karny in cotton.

Kumar (1998) reported that the plant extract of *A. paniculata* along with garlic significantly controlled aphids, thrips and mites of chilli. Kiruba and Thirunavukkarasu (2017) observed that extracts of *A. paniculata* causes 72.92 per cent deformities to *Earias vittella* (Fab.). Madihah *et al.* (2018) observed that andrographolide, an active compound of *A. paniculata* reported the highest antifeedant activity against *Plutella xylostella* (L.) larvae by disrupting the midgut histological structures. The results also revealed that andrographolide significantly reduced the invertase, amylase, protease and trypsin activity, as well as the total protein concentration of larvae of *P. xylostella*.

Widiarta *et al.* (1997) found that the crude extract of *A. paniculata* at 1600 ppm reported the highest reduction in the feeding activity of green rice leafhopper by the root immersion method and they also found the antifeedant activity of andrographolide was similar to the feeding deterrent activities of cartap and bensultap. Bernice (2000) observed that leaf extract of *A. paniculta* in combination with 2.5% neem oil emulsion and garlic at 20 g L $^{-1}$caused 40, 80 per cent deterrent effects on the aphids and epilachna bettle respectively.

Suganthy and Sakthivel (2012) reported that 2% aqueous extract of *A. paniculata* and 1% azadiracthin showed a maximum reduction of pest population of aphids, thrips, leaf miners and defoliators infesting brinjal and also conserved a greater number of natural enemies like predatory coccinellids. According to Premalatha *et al.* (2018), aqueous extract of *A. paniculata* 10 % concentration caused 42.23 per cent mortality of mites under invitro conditions at 3 days after application.

The methanol extract of *A. paniculata* showed the highest antifeedant, growth-inhibitory and oviposition deterrent activity against larval and adult stages of *Spilosoma obliqua* (Walker) (Tripathi *et al.*, 1999). Ramya and Jayakumararaj (2009) reported that aqueous leaf extracts of *A. paniculata* at a concentration of 1000 ppm showed 72.8 per cent mortality of *Helicoverpa armigera* Hubner at 24 hours after application.

The methanol and ethyl acetate extracts of *A. paniculata* at the highest concentrations (1000 ppm) caused 72.01 and 67.69 per cent adult mortality of cowpea weevil and pulse beetle respectively (Bright *et al.*, 2001). Ramya *et al*. (2011) reported that crude methanol extract of *A. paniculata* caused 83.3 per cent mortality of *H. armigera* larva. Lingampally *et al*. (2012) stated that topical application of andrographolide hinders ovary development which affects the fertility and reproductive potentiality of *Tribolium confusum* (Duval). Adekunle and Ayodele (2014) confirmed that aqueous leaf extract of *A. paniculata* caused 100 per cent mortality of *Callosobruchus maculatus* (Fabr.) at 96 hours after application. As per the findings of Vattikonda (2015) andrographolide, active compounds isolated from *A. paniculata* at a concentration of 200 ppm showed 80.05 and 83.60 per cent antifeedant activity against the fourth instar larvae of citrus butterfly at 24 and 48 hours after treatment respectively

Extracts of *A. paniculata* reduced reproductive capacity and survival of the malarial vector *Anopheles stephensi* Liston to a great extent (Kuppusamy and Murugan, 2010). Elango *et al*. (2011) observed that hexane and chloroform extract of *A. paniculata* showed 100 per cent mortality of mosquitoes (*Anopheles subpictus* Grassi). Recent evidence conclude that combined effect of *A. paniculata* and *A.lineate* Nees at 150 ppm of solvent extracts of petroleum ether: aqueous (1:1) caused 100 per cent mortality to *Culex quinquefasciatus* (Say.) and *Aedes aegypti* (Linn.) larvae after 24 h of exposure (Renugadevi *et al.,* 2013).

2.3.2.2 Bioefficacy of Oils

2.3.2.2.1 *Neem*

Khater (2012) reported that more than 100 neem formulations were found to be used for pest management worldwide. Neem based insecticides are acquired from the tree, *A. indica* belongs to the family, meliaceae (Siddiqui *et al.*, 2004).

The bitter principles of neem are mainly due to the presence of limonoids which is a group of tetranortriterpenoids of which azadirachtin is the chief active compound used in pest management (Kumar, 1998). Neem oil was found highly effective biopesticide against *Scirtothrips dorsalis* Hood (Roa *et al*., 1999), *Polyphagotarsonemus latus* (Banks)

(Venzon *et al.,* 2008) and *Aphys gosssypii* Glover (Pinto *et al.,* 2013).

Insecticidal activity of neem oil can be claimed due to repellent activity from treated plants and secondly due to antifeedant effect on the pests (Rajput *et al*., 2003). Agboka *et al*. (2009) observed ovipositional deterrence of neem oil in snout moth *Mussidia nigrivenella* Ragonot

Azadirachtin based insecticides persist only for about 4 to 8 days in the environment and therefore are immensely acceptable and appropriate for eco-friendly management of insect pests (Schmutterer, 1990). Neem seed oil 2.5 or 5% with garlic at 20 g L^{-1} controlled aphid, mite and jassid on bitter guard (KAU, 1996).

According to Ali *et al.* (2002) application of Neem Seed Kernel Extract (NSKE 5%) found to be highly effective and superior to the chemical pesticides in controlling chilli mites and thrips. The mortality of aphids in treatments with neem oil can be credited due to the presence of azadirachtin, the tetranortriterpenoid limonoid, retaining pesticidal properties According to Ghosh (2015), the botanical pesticide azadirachtin gave better results in suppressing chilli aphids (60.30 per cent).

Thamilvel (2009) inferred that application of neem oil + garlic emulsion 2 % was effective in controlling aphid. As per findings of Kumar *et al.* (2010), the application of neem oil (3.5%) was found to be highly effective in managing chilli aphid. Vinodhini and Malaikozhundan (2011) proved that the application of neem oil 80 EC 3 mL L^{-1} showed 57.72 per cent reduction of *A. gossypii* at 4 days after treatment under field conditions.

An experiment conducted by Vasantlal (2012) highlighted that neem oil (0.5%) found highly significant in reducing the thrips population. As per the research findings of Singh and Singh (2013) spraying of neem oil-based formulation at 0.03% resulted in a 33.77 per cent reduction in the mite population at1 DAT. Kumar (2016) found that neem oil 2% was most effective against thrips as it recorded the lowest population (6.90 thrips $plant^{-1}$).

Bernice (2000) reported that the application of neem oil and *Hyptis suaveolens* L. either alone or in combination were found to have high toxic and deterrent effects on aphids, brinjal shoot and fruit borer and epilachna beetle under laboratory condition. Meena and Tayde (2017) stated that neem oil 2.5 mL L^{-1} decreases the population of *S. dorsalis* to 55.78 per cent. Sundaran (2018) reported that neem oil 2% causes 50 per cent mortality of aphids 24 hours after treatment.

Sreerag and Jayprakash (2014) reported that 1% formulation with 50 mL neem oil, 20 mL cassava leaf extract and 30 mL surfactant was found to be most effective biopesticide formulation against cowpea aphid, *A. craccivora*.

An inference made by Azam (1991) showed that the neem oil 1.0 and 1.25% caused more than 80 per cent mortality of the larvae and pupae of *L. trifolii*. Ramesh and Ukey (2007) revealed that 1% neem oil was effective in reducing leaf miner infestation in tomato. As per the research findings of Rahman *et al*. (2009) application of neem oil 4% showed the highest percentage reduction (70.44 per cent infested shoots per plant) of *Leucinodes orbonalis* Guenee.

Experiments were conducted to estimate the biological efficacy of neem oil against spiraling whitefly on brinjal. Neem oil 3% showed considerably high mortality of 78.16 per cent in 10 days after treatment (Boopathi and Karuppuchamy, 2013).

Neem oil (5%) sprays were found to be effective in decreasing the populations of the chickpea pests (Rao and Srivastava, 1985; Siddappaji *et al*., 1986 and Sinha, 1993). Sontakke (1993) identified that neem oil 1% spray gave good control of *Spodoptera.* Packiam and Ignacimuthu (2012) detected that 0.6% concentration of formulation containing 85% neem oil + 15% emulsifier recorded 56.04 per cent antifeedant activity of *S. litura* within 24 HAT. As per research findings of Harish *et al.* (2014) neem 5% spray caused 88.2 per cent mortality of *Caryedon serratus* after 24 hours.

Spraying of neem oil 1.5% showed 100 per cent mortality of mustard aphid (Mani *et al.*, 1990). Neem oil at different concentrations has been proved to be effective against *B. tabaci* (Natarajan and Sundaramurthy, 1990; Rosaiah and Reddy, 1996).

The LC^{50} of an emulsifiable concentrate of neem seed oil was 0.02% against *Rhopalosiphum maidis* (Fitch) and 0.007% against *Melanaphis sacchari* (Zehntner). A 1% emulsion spray applied to sorghum in the field eliminated all the aphids in 1-2 h but showed some phytotoxicity; treatment at 0.5% was equally effective and showed no phytotoxicity, and treatment at 0.1 and 0.2% took about 48 and 24 h, respectively, to obtain the same results. When the emulsion was applied at 0.2% against *M. sacchari* in the field, no build-up of aphids on the plants for up to 3 weeks was observed. The larvae and adults of coccinellids and syrphids larvae were unaffected by any of the treatments (Srivastava and Parmar, 1985).

Kraiss and Cullen (2008) observed that both azadirachtin and neem seed oil significantly increased aphid nymphal mortality (80 and 77% respectively) while significantly increasing development time of those surviving to adulthood.

Stark and Rangus (1994) studied the effects of 'Margosan O' (MO) on the pea aphid, *Acyrthosiphon pisum* (Harris). MO significantly decreased population hike of *A. pisum* in a concentration dependent manner. At a concentration equivalent to 100 mg l^{-1} of azadirachtin, population hike was 3.5 times lower than the control. MO significantly reduced the number of molts, fecundity and longevity of *A. pisum.*

Shannag *et al.* (2014) investigated the effects of three commercial neem-based formulations, namely Azatrol (1.2% Azadiractin A and B), Triple Action Neem Oil (70% neem oil) and Pure Neem Oil (100% neem oil) on the green peach aphid, *Myzus persicae*, under both laboratory and greenhouse conditions. They found that a two-fold increase from the recommended concentration of Azatrol and Triple Action Neem Oil elicited a 50% reduction in the number of aphid s comparison with untreated leaf plants. When aphids were fed with lea ves containing neem-based insecticides, the rates of honeydew excretion were highly reduced, to 14-40% of the control, thus demonstrating feeding deterrence. Azatrol also found to have systemic action well when applied via the roots, resulting in 50% decrease in the feeding activity of treated aphids compared to that of the controls. Greenhouse evaluation of these products revealed that aphid colonization was reduced to 50-75% of the control 1 week after treatment, while a total elimination of aphids was observed by Pure Neem Oil and Azatrol when treatments were repeated 7 days after first spray.

Effectiveness of different doses of neem extracts and a synthetic organic insecticide against mustard aphid was studied by Biswas (2013) and found that neem seed extract reduced 73-81 percentage and neem leaf extracts reduced 63.16-72.55 percentage aphid population in mustard while aphid population over pre-treated plants in both the years. Among the different doses of neem extracts, the highest aphid mortality over pre-treatment (81%) was recorded from 50g neem seed per litre of water treated plots which was followed by 75g neem seed l^{-1} treated plots having reduction of 80 percentage.

Results of study conducted by Lowrey and Isman (1994) showed that neem seed oil (NSO) applied at 1.0% concentration resulted in 94 to 100 percentage mortality of second instar nymphs of lettuce aphid, *Nasonovia ribisnigri* (Mosley), and green peach aphid, *M. persicae* after nine days. The survival of adult aphids was not affected by NSO or AZA, but the survival of next generation from treated adult *M. persicae* and *N. ribisnigri* was decreased significantly. The lethal concentration of AZA resulting in 50% mortality of second instar nymphs of nine species of aphids ranged from to 635.0 ppm for strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell), on strawberry and 2.4 ppm for *M. persicae* on pepper.

Investigations were conducted on *Encarsia formosa* Gahan parasitoid of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood. Results revealed that the application of 10 ppm azadirachtin was relatively nontoxic, whereas the concentration of 20 ppm led to a low but significant reduction of the fitness of *E. formosa* (Feldhege and Schmutterer, 1993).

The toxic effect of neem seed kernel extracts prepared with different solvents against the predatory spider, *Chiracanthium mildei* L. Koch, was investigated and the order of toxicity of the 4% extracts was pentane < acetone < ethanol << methanol = water (nontoxic). All extracts were nontoxic at 2.5% (Mansour *et al.*, 1986).

Mohan *et al.* (2007) reported that the effect of combined treatment of neem with Beavaria *bassiana* in comparison to single treatments with either of them on *S. litura* was tested in laboratory bioassays. The combination treatment was found to have synergistic effect on insect death when *B. bassiana* isolate compatible with neem was used, while, with an isolate sensitive to neem, an antagonistic effect was observed.

The effects of Margosan-O, Azatin and RD9-Repelin on the phytophagous mite *Tetranychus cinnabarinus* (Boisd.), the predacious mite *Typhlodromus athiasae* Porath and Swirski, and the predatory spider *C. mildei* were studied and compared under invitro condition. Neem formulations does not exhibit any toxic effect on *C. mildei.* Margosan-O and Azatin were not toxic to either *T. cinnabarinus* or *T. athiasae*, but RD9-Repelin was found to be toxic to both the phytophagous and the predacious mite (Mansour *et al.*, 1993).

Stark (2013) reported that spiders are susceptible to the pesticides used to control pest insect species. Though, botanical insecticides, especially those derived from the neem tree, have been shown to be less toxic to spiders than synthetics.

A study was carried out by Radhika and Sahayaraj (2014) to determine the synergistic effects of neem oil, vijayneem, pungam oil, and biosilver nanoparticles (10, 20, 30, 40 and 50%) blended with monocrotophos and screend against third instar larvae of tobacco caterpillar by dermal toxicity bioassay. The dermal toxicity bioassay reported 100% mortality in monocrotophos + neem oil and monocrotophos + biosilver nanoparticles in 10% on eighth day, monocrotophos + pungam oil in 20% on second day and monocrotophos + vijayneem in 50% at ninth day. The highest growth was observed in neem oil and biosilver nanoparticles when mixed with monocrotophos.

2.3.2.2.2. *Pongamia*

Pongamia pinnata (L.) Panigrahi belonging to the family Leguminosae is an abundant source of flavonoids, chalcones, steroids and terpenoids. The oil serves as defensive agents against insect pests (Pavela, 2004; Pavela *et al.*, 2005).

Rao and Dhingra (1997) observed karanjin, an active compound isolated from pongamia seed oil showing juveno-mimetic activity in the larvae of *Tribolium castaneum* (Herbst).

An experiment was conducted by Kumar and Singh (2002) and proved that the persistence of pongamia oil is greater than other botanical insecticides. Pongamia oil also express a good synergistic effect with a number of chemical pesticides and also recorded greater biological activity. Increasing potential as a biopesticide is due to its antifeedant, ovicidal, juvenile hormone activity and oviposition deterrent activity that can be attributed by karanjin, the major flavonoid of the seed oil.

Meera *et al.* (2003) highlighted active components of the karanjin group, extracted in water from pongamia oil cause poisonous effects on *S. litura* larvae. Reddy and Kumar (2006) highlighted pongamia oil 1% gave suitable control of *P. latus*. Pongamia oil at 1% concentration found to be highly effective in reducing chilli thrips (Vasanthalal, 2012). Kaur and Singh (2013) observed that 1% pongamia soap gave significantly better control of thrips.

Meena and Tayde (2017) reported that pongamia oil 4% reduces the population of *S.dorsalis* to 55.64 per cent. Veena *et al*. (2017) stated that pongamia oil 2 mL L^{-1} caused 26.67, 36.67, 46.67 and 56.76 per cent mortality of *P. latus* 24, 48, 72 and 96 hours after treatments (HAT) under laboratory condition.

Jothi *et al.* (1990) assessed the various oils of plant origin and their extracts against citrus aphids, based on the cost and effectiveness of treatments. They found pongamia seed extract (2%) and pongamia oil (1%) was effective in controlling citrus aphid to a great extent. Spraying of pongamia oil 80 EC 3 mL L^{-1} recorded a 40.58 per cent reduction of *A. gossypii* three days after treatment under field conditions (Vinodhini and Malaikozhundan, 2011).

Arya (2015) reported that oxuron a commercial botanical product comprising of neem oil and karanja oil was found to be effective against sucking pests of brinjal. Sundaran (2018) also stated that oxuron was found to be effective against *A. gossypii*.

Packiam and Ignacimuthu (2012) mentioned 0.6% concentration of formulation containing 85% pongamia oil + 15% emulsifier recorded 51.06 per cent antifeedant activity in tobacco caterpillar 24 hours after treatment application. PONNEEM a commercial botanical product comprising of neem oil and karanja oil in the ratio of (1:1) showed 58.16 per cent mortality against American boll worms (Packiam *et al.*, 2013).

As per research findings of Harish *et al.* (2014) spraying of 5% pongamia oil caused 37.4 per cent mortality of *C. serratus* 24 HAT. Stepanycheva *et al.* (2014) observed pongamia oil 1% did not impart any negative influence on insect pollinators of hymenopterans, lepidoptera, hemiptera, coleoptera and diptera.

Karanja oil (2%) was reported to prolong larval development and growth inhibiting activity of American boll worm (Bajpai and Sehgal, 1994). As per the research findings of Rahman *et al.* (2009) 4% pongamia oil showed a maximum percentage reduction of brinjal shoot and fruit borer.

2.4 PHYTOTOXICITY STUDIES

Madiwalar in 2015 Conducted a study to assess the level of phytotoxicity of botanical insecticides (*A. indica*, *P. pinnata*, *Calotropis gigantea* (L.) Dryland, *Vitex negundo* L., *Ricinus communis* L., *Clerodendrum inerme* (L.) Gaertn., *Parthenium hysterophorus* L., linseed oil and sunflower oil) and 17 chemical insecticides to sweet sorghum. Data were recorded on phytotoxicity symptoms, such as chlorosis, bronzing and white blotch. Quinalphos exhibited the highest phytotoxicity symptoms compared to all the other chemical insecticide treatments on sorghum leaves. All the plants treated with botanical insecticides did not exhibit any phytotoxicity responses.

Buss and Park-Brown (2002) stated that most botanicals are not phytotoxic since they suddenly break down or are metabolized by enzymes inside bodies of their target pests. Breakdown may occur rapidly, so that the insecticide only temporarily stuns the insect, and do not kill.

Study by Xuan *et al.* (2004) documented that neem strongly inhibits germination and growth of several specific crops: rice (*Oryza sativa* L.), alfalfa (*Medicago sativa* L.), bean (*Vigna angularis*) carrot (*Daucus carota* L.), radish (*Raphanus sativus* L.)and sesame (*Sesamum indicum* L.) and weeds: *Echinochloa crusgalli, Monochoria vaginalis* and *Aeschynomene indica* L. in a bioassay and in soil.

Nimbolide B (**1**) and nimbic acid B (**2**) are two active principles in neem leaves. Nimbolide B restricted the growth of cress and barnyard grass at concentrations greater than 0.1- 3.0 μM. Nimbic acid B hindered the growth of cress and barnyard grass at concentrations greater than 0.3–1.0 μM. These results prove that that nimbolide B and nimbic acid B may contribute to the allelopathic effects caused by neem leaves (Kato-Noguchi *et al.,* 2014).

Laboratory experiments conducted by Sreedharan *et al.* (2015) reported that mineral oil in combination with either neem oil or *Pongamia glabra* seed oil is effective in checking the whitefly population; furthermore, it is not phytotoxic and is safer for its common predator green lace wing.

Materials and methods

3. MATERIALS AND METHODS

The investigation was carried out in College of Agriculture Vellayani during the period 2019-20. Location of the experimental site is 8° 25' North latitude and 76° 59' East longitude at an altitude of 29 meters above mean sea level (MSL). In the experimental site predominant soil is laterite belonging to Vellayani series. Texture in the experimental site is sandy clay loam with acidic nature.

3.1 DEVELOPMENT OF BIOPESTICIDE

3.1.1 MAINTAINING *A. paniculata* FIELD

Seeds of *A. paniculata* were obtained from Aromatic and Medicinal Plants Research Station (AMPRS), Odakkali under Kerala Agricultural University. Seeds were soaked in GA3 (500 ppm) over night and sowed in a portray filled with cow dung, sand and coir pith mix and kept under a polyhouse. Two-week-old seedlings were planted in the nursery beds in early September. From nursery, 6-week-old healthy seedlings were transplanted in the main field.

3.1.1.1 Preparation of experimental plot

Experimental plot was brought to fine tilth by ploughing accompanied by tilling and levelling. Organic manures and fertilizers were applied and given 4-6 light irrigations until harvest.

3.1.1.2 Planting method

Broad beds and furrows were taken at 15 cm spacing. Seedlings were planted in beds at 30 cm spacing

3.1.1.3 Fertilizer application

Apart from organic manures applied as basal dose, *Andrographis* requires major nutrients such as N, P and K through Urea, Rajphos and MOP respectively. These fertilizers were applied in two splits at an interval of 1 month to obtain 75:75:50 kg NPK/ha. For the effective absorption by plants, fertilizers were applied in basin and mixed with soil thoroughly. Irrigation should provide before or after the fertilizer application.

3.1.1.4 Irrigation method

On the basis of weather and soil condition irrigation schedules was fixed. 4-6 light irrigations are entailed till harvest

3.1.1.5 Intercultural operations

Two or three weeding is essential to check weed growth especially during monsoon season viz. 20 days and 60 days after transplanting.

3.1.2 IN VITRO EVALUATION OF DIFFERENT FORMULATIONS OF *A. paniculata*

3.1.2.1 Development of formulations

The basic components used for developing the formulations were

1. Plant extract of *A. paniculata*
2. Oils *viz.*, neem oil and pongamia oil
3. Surfactant., Triton X-100

3.1.2.2 Preparation of plant extract

Tender stems, leaves, flowers and roots of *A. paniculata* maintained in Instructional farm at College of Agriculture, Vellayani, were washed thoroughly with clean water and chopped into approximately 0.5 cm size for easy grinding. Fresh chopped plant material of *A. paniculata* (2.5 kg) was macerated in an electric grinder without adding water to get 1 litre of the plant extract. The extract obtained was first filtered through strainer to remove debris then again sieved through double layered muslin cloth to get a clear plant extract.

3.1.2.3 Preparation of oil-based formulations of *A. paniculata*

Effective combination of Extract-oil-surfactant (EOS) were prepared using *A. paniculata* extract, oils and Triton X-100. After mixing the components in the desired ratio, the formulations were kept in magnetic stirrer at 400 rpm for 30 minutes for proper mixing to get a better emulsion. Formulations after preparation were tested for blooming and emulsion stability test (BIS, 1997; Allawzi *et al.,* 2016).

3.1.2.4 Maintenance of stock culture of test organism

***A. craccivora* Koch**

The species of aphid that was taxonomically confirmed as *A. craccivora,* was reared on cowpea variety Geethika. Seedlings were transplanted to plastic pots of 500 mL filled with potting media containing sand, soil and farmyard manure in the ratio of 1:2:1. At two to three leaf stages, aphids were released into seedlings using camel paint brush. After inoculation, seedlings were maintained in rearing cage for multiplication.

3.1.2.5 Screening of different oil-based formulations of *A. paniculata*

Screening of different oil-based formulations of *A. paniculata* was carried out in laboratory using *A. craccivora* as test insect. Concentrations of 1, 2, 3 and 5% of the following treatments were tested against the test insect. The treatments were applied using hand sprayer. Twenty aphids were placed in each live plant and directly sprayed with 10 mL formulations.

Design: CRD   Treatments: 21   Replications: 3

1. Plant extract of *A. paniculata* + neem oil +Triton X-100 (7:2:1).
2. Plant extract of *A. paniculata* + pongamia oil +Triton X-100 (7:2:1).
3. Plant extract of *A. paniculata* + Triton X-100 (9:1).
4. Neem oil + Triton X-100 + Water (2:1:7) (Check)
5. Pongamia oil + Triton X-100 + Water (2:1:7) (Check)

(1, 2, 3 and 5% of the above combinations)

21. Untreated control (Water alone)

The treated plants were then covered and secured with plastic bags in order to prevent the migration of aphids between the treated plants. The number of dead aphids were counted at 1,3,5 and 7 days after treatment (DAT) following a regular time. The percentage mortality was calculated and the cumulative percentage mortality was statistically analyzed.

a) *A. paniculata*

b) Flowers and pods

c) Chopped plant parts

d) Weighing process

Plate 1: Preparation of plant extract

a) Magnetic stirrer

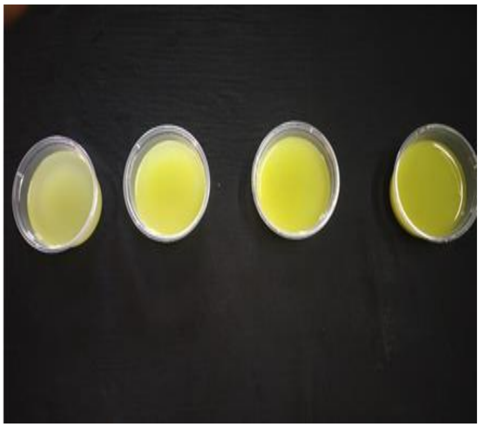
b) Refrigeration of formulations

c) Exising aphid colonies

d) Aphid Rearing

Plate 2: Preparation of oil based formulations

a) Host plant inoculation



b) 1, 2, 3 and 5% formulations

c) Treated plants

d) Secured with plastic bags

Plate 3 : Screening of effective formulations

3.2 EVALUATION OF EFFECTIVE FORMULATIONS

A pot culture experiment was conducted to evaluate the efficacy of selected treatments for the management of sucking pest complex of cowpea. Two promising *A. paniculata* based treatments selected from lab experiment were evaluated for their effectiveness in field conditions. The above-mentioned treatments were selected for further evaluation with thiamethoxam 25% WG and neem oil 2% as check.

Seeds of cowpea variety Geethika were procured from Department of Vegetable science, College of Agriculture, Vellayani, and were kept soaked overnight and sowed in a protray filled with cowdung, sand and coir pith mix and kept it under a poly house. Two-week-old healthy seedlings with 4 to 6 leaf stage were transplanted to grow bags (40 x 24 x 24 cm) filled with the potting mixture prepared with sand, soil and farmyard manure in 1:2:1 ratio. The crop was raised following the package of practices recommendations of Kerala Agricultural University (KAU, 2016).

Plot was brought to fine tilth by repeated ploughing followed by tilling and levelling and layout of the field was done.

Design: CRD   Treatments: 13 Replications: 3

T1 1% formulation

T2 2% formulation

T3 3 % formulation

T4 5% formulation

T5 6 % formulation

(Of two promising *A. paniculata* based treatments)

T11 Thiamethoxam 25 % WG 50 g a.i ha^{-1}

T12 Neem oil 2%

T13 Untreated control (Water alone)

A consistent population of sucking pests were maintained in plants by avoiding plant protection interventions. After recording the pretreatment population of the sucking pests, the first round of treatments was applied at 30 days after transplanting (DAT) in the vegetative stage of the crop. Treatments were applied to the entire plant using a hand sprayer ensuring coverage of both abaxial and adaxial surfaces of leaves. Post treatment population of sucking pests was recorded on 1, 3, 5 and 7 days after spraying (DAS). All the pre and post treatment observation was taken early in the morning. From each plant, number of aphids present in an aphid colony occupying in a 10 cm length of shoot was counted and multiplied with total number of such colonies to get an estimation of total population of aphids per plant.

The number of natural enemies, pollinators and neutrals seen in the plants were counted and mean value were calculated one day before pre-treatment and 1, 3, 5 and 7 DAS.

3.2.2 Growth parameters of cowpea pods treated with different formulations

The following growth parameters were recorded.

a. Number of pods

b. Pod length

c. Pod weight

d. Number of seeds per pod

Length of the pod from the base to the tip were measured and expressed in centimeter (cm). Weight of pods in a single harvest was measured and expressed in grams per plant (g plant-1). Number of pods and number of seeds per pod at the bearing stage was taken into account.

3.2.3 Phytotoxicity studies

1, 2, 3, 5, 10 and 20 % concentration of all oil-based formulations were sprayed over cowpea seedlings and observation for symptoms like yellowing, scorching, necrosis, epinasty and hyponasty were done. Symptoms were graded based on per cent injured as prescribed by CIBRC (Central insecticides board and registration committee) grade scale at every 1, 3, 5- and 7-days interval.

Percentage of phytotoxicity	CIBRC Grade
No phytotoxicity	0
1-10	1
11- 20	2
21-30	3
31-40	4
41-50	5
51-60	6
61-70	7
71-80	8
81-90	9
91-100	10

3.3 STATISTICAL ANALYSIS

The data collected from the laboratory and field experiments were subjected to statistical analysis using WASP software. Data were analyzed using one-way analysis of variance after subjected to angular and square root transformations appropriately.

a) Land levelling

b) Field at 30 DAP

Plate 4: Field study

Result

3. RESULT

4.1 IN VITRO EVALUATION OF DIFFERENT FORMULATION OF *A. paniculata*

4.1.1 Development of Formulations

Formulations were prepared by mixing extractant, oil and surfactant in the ratio 7:2:1

4.1.2 Efficacy of Oil Based Formulation of *A. paniculata* against *A. craccivora*

Different concentrations (1, 2, 3 and 5%) of oil-based formulation of *A. paniculata* were evaluated against *A. craccivora*. The data on per cent mortality of aphids are presented below.

Results of data on mortality of *A. craccivora* treated with oil-based formulations of *A. paniculata* at 1% concentration is depicted in Table1. Highest mortality was recorded for extract of *A. paniculata* + neem oil + Triton X-100 (75%) at 1 DAT. This was found to be on par with extract of *A. paniculata* + pongamia oil + Triton X-100 and Neem oil + Triton X-100 which recorded 71.67 and 65 percentage mortality respectively. The next highest mortality was recorded for extract of *A. paniculata* + Triton X-100 (48.33) and it was on par with Pongamia oil + Triton X-100 which recorded percentage mortality of 38.33.

On 3rd DAT, extract of *A. paniculata* + neem oil + Triton X-100 showed superiority over other treatments with 95.00 percent mortality. This was followed by extract of *A. paniculata* + pongamia oil+ Triton X-100 with 88.33 percent mortality. This was again followed by Neem oil +Triton X-100 with 81.67 percent mortality. *A. paniculata* + Triton X-100 showed 71.66 percent mortality though inferior to rest of the treatments. Least mortality (0.00%) was observed for control.

On 5thDAT, extract of *A. paniculata*+ neem oil + Triton X-100 showed best results with 100 percent mortality. The superior treatment was followed by extract of *A. paniculata* + pongamia oil + Triton X-100 with 95 percent mortality. This was found to be on par with Neem oil + Triton X-100 with 93.33 percent mortality. The next highest percentage mortality was observed for extract of *A. paniculata* + Triton X100 (85%). Pongamia oil + Triton X-100with 76.67 percent mortality showed significant superiority overcontrol (0.00%).

On 7th DAT, extracts of *A. paniculata* + neem oil + Triton X-100, *A. paniculata* +

pongamia oil+ Triton X-100 and Neem oil +Triton X-100 recorded highest mortality (100%). This was followed by extract of *A. paniculata* + Triton X-100 (93.33%). Pongamia oil + Triton X-100recorded 90.00% and was superior to control (0.00%).

Results of data on mortality of *A. craccivora* treated with oil-based formulations of *A. paniculata* at 2% concentration is inscribed in Table 2. On 1st DAT, extracts of *A. paniculata* + neem oil + Triton X-100 showed highest percent mortality of 91.67. This was found to be on par with extracts of *A. paniculata*+ pongamia oil+ Triton X-100 with 88.33 percent mortality. This was followed by Neem oil + Triton X-100 as well as *A. paniculata*+ Triton-X-100. Both treatments were found to be on par with each other with 75.00 and 68.33 percent mortality respectively. Pongamia oil + Triton X-100 exhibited 50.00 percent mortality and control recorded 0.00 percent mortality.

Upon 3rd DAT, extracts of *A. paniculata* + neem oil+Triton X-100recorded highest percent mortality (100%). This was found to be on par with extracts of *A. paniculata*+ pongamia oil+ Triton X-100 having 96.67 percent mortality and Neem oil + Triton X-100 having 95 percent mortality. The next highest percent mortality was observed for extract of *A. paniculata* +Triton X-100 (85%). Pongamia oil+Triton X-100 recorded 68.33 percent mortality and was superior to control (5%).

On 5thDAT, extracts of *A. paniculata* + neem oil + Triton X-100 and *A. paniculata* + pongamia oil+ Triton X-100 recorded highest percent mortality (100 %). This was found to be on par with Neem oil +Triton X-100 and extract of *A. paniculata* + Triton X100 recording 98.33 percent mortality. This was followed by Pongamia oil + Triton X-100 with 91.67 percent mortalityand was found to be statistically superior with control recording 8.33 percent mortality.

On 7thDAT, treatments did not vary significantly. All the treatments except control were found to be highly effective with mortality percent ranging from 98.00 to 100.00.

Perusal of data presented in the Table 3 revealed the following findings on the mortality of *A. craccivora* as influenced by oil-based formulations of *A. paniculata* at 3% concentration.

On 1stDAT, the highest percent mortality (98.33%) was recorded for *A.paniculata* + neem oil + Triton X-100 and it was significantly different from all other treatments. This was followed by extract of *A. paniculata* + Pongamia oil+ Triton X-100 recording 95.00 percent mortality. Neem oil + Triton X-100 recorded 88.33 percent mortality and this was found to be statistically on par with extract of *A. paniculata* + Triton X100 recording 83.33 percent mortality. The next highest mortality was observed for Pongamia oil + Triton X-100 (73.34). All the above treatments showed significant superiority over control which recorded percent mortality of 16.67%.

On 3rd DAT, *A. paniculata* + neem oil + Triton X-100, *A. paniculata* + pongamia oil+ Triton X-100 and Neem oil + Triton X-100 showed superiority over other treatments with 100.00 percent mortality. This was followed by Pongamia oil+ Triton X-100 and extract of*A. paniculata*+ Triton X100 recording 95 percent mortality. Untreated plants recorded significantly lowest percent mortality (5%).

On 5th and 7th DAT, all treatments showed superior results with 100 percent mortality and untreated check recorded 18.33 percent mortality. On 7th DAT similar trend was observed except for control recording 6.67 percent mortality.

Results of data on mortality of *A. craccivora* treated with oil-based formulations of *A. paniculata* at 5% concentration is illustrated in Table 4.On 1st DAT, extracts of *A. paniculata* + neem oil + Triton X-100, *A. paniculata*+ pongamia oil+ Triton X-100 and Neem oil + Triton X-100 recorded 100.00 percent mortality and was found to be statistically superior to *A. paniculata* + Triton X100 and Pongamia oil+ Triton X-100 recording 90.00 percent mortality. Least mortality was observed for untreated plants (8.33%)

On the 3rd and 5th day of treatment, all the treatments except control showed 100.00 percent mortality which did not vary significantly from other treatments. Untreated check recorded least mortality of 11.67 %.

Upon 7th DAT, extracts of *A. paniculata* + neem oil + Triton X-100, *A. paniculata* + pongamia oil+ Triton X-100, Neem oil + Triton X-100 and Pongamia oil+ Triton X-100 recorded 100.00 percent mortality. This was found to be on par with extracts of *A. paniculata* + Triton X100 recording 98.33 percent mortality. Untreated plants recorded least mortality (18.33%).

4.2 EVALUATION OF EFFECTIVE FORMULATION FOR FIXING THE DOSE.

Based on the laboratory evaluation two treatments which have given good results were selected for further studies

1. Plant extract of *A. paniculata* + neem oil + Triton X-100.

2. Plant extract of *A. paniculata* + pongamia oil + Triton X-100.

The above-mentioned treatments were selected for further evaluation in field with thiamethoxam 25% WG and neem oil 2% as check.

4.2.1 Effect of Oil Based Formulations of *A. paniculata* on the population of sucking pests in the Vegetative Stage of the Cowpea

Population of cowpea aphid (*A. craccivora*) subsequent to the application of treatments undertaken at 30 days after planting (DAP) were recorded at 1, 3, 5 and 7 days after treatment (DAT). Mean population of cowpea aphid at different intervals after treatment application are presented in the Table 5.

At 1 DAT, all the treatments were effective in reducing the aphid population in crop compared to the untreated (168.00 per plant). The treatment combinations, *A. paniculata* + neem oil + Triton X-100, *A. paniculata*+ pongamia oil + Triton X-100 at 6% concentration and thiamethoxam 25% WG significantly reduced aphid count (0.00 per plant) and found statistically on par with 5% concentration of *A. paniculata*+ pongamia oil + Triton X-100(10.64 per plant) and 3 and 5% concentration of Neem oil + Triton X-100 (34.64 per plant and 24.00 per plant respectively).

At 3 DAT, there was statistically significant difference in number aphids recorded in different treatments. All the treatments significantly reduced number of aphids in cowpea compared to untreated (200.00 per plant).3,5and 6% concentration of neem-based formulation, 6%concentration of pongamia based formulation, neem oil 2% and thiamethoxam 25% WG showed superiority over other treatments (0.00 per plant). This was followed by 5% concentration of pongamia based formulation as well as2% concentration of neem-based formulation which were found to be statistically similar (5.20 and 5.36 per plant respectively). Aphid population showed significant reduction in plants sprayed with similar in plants sprayed with 3 concentration of pongamia based formulation (13.28 per plant).

At 5 DAT, there was no significant difference between treatments except control (253.36 per plant). The lowest population of aphids was recorded with 2, 3, 5 and6% concentration of neem-based formulation as well as 3, 5 and 6% concentrations of pongamia based formulation, neem oil 2% and thiamethoxam 25% WG (0.00 per plant) which were found to be on par with each other. This was followed by 2% pongamia based formulation which recorded a least mean population of 5.36 aphids per plant. Neem based formulations at 1% exhibited significant lower population (74.64 per plant) and 1% pongamia based formulation recorded population of 138.64 per plant though inferior to rest of the treatments.

On the 7thday of spraying, the aphid population showed the same trend as in the 5thday where treatments with 2,3,5 and 6% concentration of both neem based and pongamia based formulations as well as thiamethoxam 25% WG were statistically similar (0.00 per plant). This was followed by 1% neem-based formulation recording mean population of 112.00 per plant. Pongamia based formulation at 1% recorded significantly lower population (138.64 per plant) compared to control plot (260.00 per plant). Neem oil 2% check recorded recurrence of aphids.

Table 1. Mortality of *A. craccivora* with oil-based formulation of *A. paniculata* at 1% concentration

Treatments	Mortality (%) Days after treatment (DAT)			
	1	3	5	7
P E A (70%) + neem oil (20%) + T (10 %)	75.00 (60.47)[a]	95.00 (77.08)[a]	100.00 (89.36)[a]	100.00 (89.36)[a]
P E A (70%) + pongamia oil (20 %) + T (10%)	71.67 (58.26)[a]	88.33 (70.50)[b]	95.00 (77.08)[b]	100.00 (89.36)[a]
P E A (90%) + T (10 %)	48.33 (44.04)[b]	71.66 (57.86)[d]	85.00 (67.21)[c]	93.33(75.24)[b]
Neem oil (20%) + T (10%)+ Water(70%)	65.00 (53.76)[a]	81.67 (64.69)[c]	93.33 (75.24)[b]	100.00 (89.36)[a]
Pongamia oil (20%) + T (10 %)+ Water(70%)	38.33 (38.22)[b]	56.67 (48.83)[e]	76.67 (61.14)[d]	90.00 (71.56)[c]
Control	0.00 (0.64)[c]	0.00 (0.64)[f]	0.00 (0.64)[e]	0.00 (0.64)[d]
CD (0.05)	(6.05)	(6.61)	(1.58)	(8.05)

(Values in the parenthesis are angular transformed) PEA: Plant extract of *A. paniculata*
T: Triton X-100 DAT: Days after treatment

Table 2. Mortality of *A. craccivora* with oil-based formulation of *A. paniculata* at 2% concentration

Treatments	Mortality (%)			
	1 DAT	3 DAT	5 DAT	7 DAT
P E A (70 %) + neem oil (20 %) + T (10 %)	91.67 (73.403)[a]	100.00 (89.36)[a]	100.00 (89.36)[a]	100.00 (89.36)[a]
P E A (70 %) + pongamia oil (20 %) + T (10 %)	88.33 (70.502)[a]	96.67 (81.17)[a]	100.00 (89.36)[a]	100.00 (89.36)[a]
P E A (90 %) + T (10 %)	68.33 (55.77)[b]	85.00 (67.21)[b]	98.33 (85.26)[a]	100.00 (89.36)[a]
Neem oil (20 %) + T (10%)+ Water(70%)	75.00 (60.00)[b]	95.00 (77.08)[a]	98.33 (85.26)[a]	98.33 (85.266)[a]
Pongamia oil (20 %) + T (10 %)+ Water(70%)	50.00 (45.00)[c]	68.33 (55.77)[c]	91.67 (73.40)[b]	98.33 (85.266)[a]
Control	0.00(0.64)[d]	5.00 (8.02)[d]	8.33 (13.95)[c]	1.67 (4.73)[b]
CD (0.05)	(4.91)	(10.69)	(11.44)	(8.92)

(Values in the parenthesis are angular transformed) PEA: Plant extract of *A. paniculata*

T: Triton X-100   DAT: Days after treatment

Table 3. Mortality of *A. craccivora* with oil-based formulation of *A. paniculata* at 3% concentration

Treatments	Mortality (%)			
	1 DAT	3 DAT	5 DAT	7 DAT
P E A (70 %) + neem oil (20 %) + T (10 %)	98.33 (85.27)[a]	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]
P E A (70 %) + pongamia oil (20 %) + T (10 %)	95.00 (77.08)[b]	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]
P E A (90 %) + T (10 %)	83.33 (65.95)[c]	95.00 (77.08)[b]	100.00(89.36)[a]	100.00(89.36)[a]
Neem oil (20 %) + T (10%)+ Water(70%)	88.33 (70.12)[c]	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]
Pongamia oil (20 %) + T (10 %)+ Water(70%)	73.34 (58.93)[d]	95.00 (77.08)[b]	100.00(89.36)[a]	100.00(89.36)[a]
Control	16.67 (24.05)[e]	5.00 (10.67)[c]	18.33 (25.31)[b]	6.67 (12.12)[b]
CD (0.05)	(6.05)	(6.61)	(1.58)	(8.05)

(Values in the parenthesis are angular transformed) PEA: Plant extract of *A. paniculata* *T*: Triton X-100   DAT: Days after treatment

Table 4. Mortality of *A. craccivora* with oil-based formulation of *A. paniculata* at 5% concentration

Treatments	Mortality (%)			
	1 DAT	3 DAT	5 DAT	7 DAT
P E A (70 %) + neem oil (20 %) + T (10 %)	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]
P E A (70 %) + pongamia oil (20 %) + T (10 %)	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]
P E A (90 %) + T (10 %)	90.00 (71.56)[b]	100.00(89.36)[a]	100.00(89.36)[a]	98.33 (85.50)[a]
Neem oil (20 %) + T (10%)+ Water(70%)	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]
Pongamia oil (20 %) + T (10 %)+ Water(70%)	90.00 71.56)[b]	100.00(89.36)[a]	100.00(89.36)[a]	100.00(89.36)[a]
Control	8.33 (16.59)[c]	11.67 (18.61)[b]	11.67 (19.49)[b]	18.33 (25.00)[b]
CD (0.05)	(2.31)	(7.16)	(4.13)	(4.31)

(Values in the parenthesis are angular transformed) PEA: Plant extract of *A. paniculata*
T: Triton X-100   DAT: Days after treatment

Table 5: Effect of oil-based formulations of *A. paniculata* on *A. craccivora*

Treatments	Dose (%)	Precount	Mean population (Number plant^{-1}) *			
			1 DAT	3 DAT	5 DAT	7 DAT
PEA (70 %) + neem oil (20 %) + Triton X-100 (10 %)	1	93.36 (9.66)	66.64 (8.19)^{c}	29.36 (5.46)^{c}	74.64 (8.69)^{c}	112.00 (10.67)^{c}
	2	112.00 (10.58)	50.72 (7.15)^{e}	5.36 (2.42)^{f}	0.00 (0.71)^{d}	0.00 (0.71)^{e}
	3	130.64 (11.43)	34.64 (5.92)^{g}	0.00 (0.71)^{g}	0.00 (0.71)^{d}	0.00 (0.71)^{e}
	5	96.00 (9.79)	24.00 (4.95)^{h}	0.00 (0.71)^{g}	0.00 (0.71)^{d}	0.00 (0.71)^{e}
	6	128.00 (11.31)	0.00 (0.71)^{j}	0.00 (0.71)^{g}	0.00 (0.71)^{d}	0.00 (0.71)^{e}
PEA (70 %) + pongamia oil (20 %) + Triton X-100 (10 %)	1	94.24 (9.70)	94.24 (9.70)^{b}	112.00 (10.58)^{b}	138.61 (11.79)^{b}	138.64 (11.11)^{b}
	2	112.00 (10.58)	56.00 (7.51)^{d}	24.00 (4.95)^{d}	5.36 (2.42)^{d}	0.00 (0.71)^{e}
	3	133.36 (11.54)	48.03 (6.96)^{f}	13.28 (3.71)^{e}	0.00 (0.71)^{d}	0.00 (0.71)^{e}
	5	146.64 (12.11)	10.64 (3.33)^{i}	5.20 (2.38)^{f}	0.00 (0.71)^{d}	0.00 (0.71)^{e}
	6	189.36 (13.76)	0.00 (0.71)^{j}	0.00 (0.71)^{g}	0.00 (0.71)^{d}	0.00 (0.71)^{e}

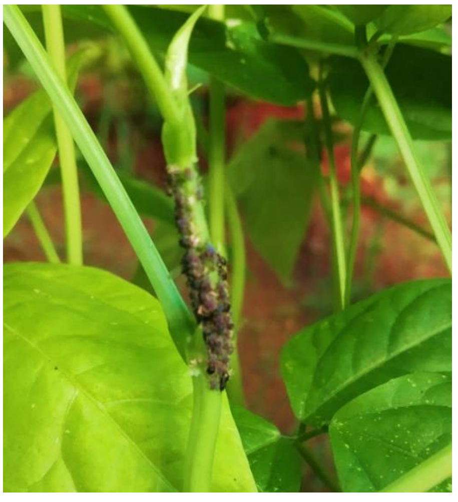
Table 5 (continues). Effect of oil-based formulations of *A. paniculata* on *A. craccivora*

Treatments	Dose (%)	Precount	Mean population (Number plant^{-1}) *			
			1 DAT	3 DAT	5 DAT	7 DAT
Thiamethoxam 25 % WG	50 g a.i ha^{-1}	304.00 (17.43)	0.00 (0.71)^{j}	0.00 (0.71)^{g}	0.00 (0.71)^{d}	0.00 (0.71)^{e}
Neem oil	2	120.00 (10.95)	53.36 (7.34)^{d}	0.00 (0.71)^{g}	0.00 (0.71)^{d}	40.00 (6.36)^{d}
Control	Nil	154.64 (12.43)	168.00 (12.98)^{a}	200.00 (14.16)^{a}	253.36 (15.93)^{a}	260.00 (16.17)^{a}
CD (0.05)		NS	(12.08)	(8.56)	(6.16)	(5.28)

Mean of 3 replications comprising 3 plants each (Values in the parenthesis are square root transformed) DAT: Days after treatment PEA: Plant extract of *A. paniculata*

a) Damaging symptoms

b) Post treatment population



c) Pretreatment population

Plate 5: *Aphis craccivora*

4.2.2 Safety Evaluation of Oil based formulations of *A. paniculata* on Natural Enemies and ants associating with aphids in Cowpea Ecosystem.

The results of evaluation of oil-based formulation of *A. paniculate* on the safety of natural enemies of pests in cowpea ecosystem are furnished in Table 6 to 9. The count of predators such as, coccinellids, spiders and ants associated with aphids per plant were taken 1, 3, 5 and 7 days after spraying.

4.2.2.1 Coccinellid beetles

Coccinellid beetles encountered in cowpea ecosystem were *Coccinella transversalis*, *Chilomenus sexmaculata* and *Chilocorus* sp. Mean population of coccinellid beetles after spraying at vegetative phase is illustrated in Table 6.

At 1 and 3 DAT, untreated plants recorded significantly higher number of coccinellids (1.67 per plant) than the treatments. The mean population of coccinellid beetles was found to be on par with each other among rest of treatments with population ranging from 0.00 to 0.33 per plant.

At 5 DAT, significant higher number of coccinellids was recorded in control plots (2.00 per plant). The mean population of coccinellid beetles ranged from 0.00 to 0.66 per plants for remaining treatments. Similar trend was observed during 7th DAT except for neem oil 2% which showed re-establishment of beetles.

4.2.2.2 Syrphids

Mean population of syrphids after spraying at vegetative phase is illustrated in Table 7. There was no significant difference between treatments up to 3 DAT regarding population of predatory syrphids.

At 5 DAT, maximum population of syrphids was observed in control plots as well as 1% concentration of neem-based formulation (1.00 and 0.67 respectively). No syrphid population was recorded for chemical check thiamethoxam 25% WG, 3, 5 and 6% concentration of neem based and pongamia based formulations as well as 2 % neem oil. A similar trend was observed in 7th DAT except for neem oil 2% that reported reoccurrence of syrphids.

Table 6. Effect of oil-based formulation of *A. paniculata on* coccinellids in cowpea

Treatments	Dose (%)	Precount	Mean population (Number plant^{-1}) * Days after treatment			
			1	3	5	7
Plant extract of *A. paniculata* (70 %) + neem oil (20 %) + Triton X-100 (10 %)	1	0.00(0.71)	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{c}
	2	0.00(0.71)	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{c}
	3	0.67(1.05)	0.33(0.88)^{b}	0.33(0.88)^{b}	0.33(0.88)^{b}	0.33(0.88)^{bc}
	5	0.67(1.05)	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{c}
	6	1.33(1.29)	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{c}
Plant extract of *A. paniculata* (70 %) + pongamia oil (20 %) + Triton X-100 (10 %)	1	0.33(0.88)	0.33(0.88)^{b}	0.33(0.88)^{b}	0.66(0.99)^{b}	0.66(0.99)^{bc}
	2	0.00(0.71)	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{c}
	3	0.67(1.05)	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{c}
	5	0.67(1.05)	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{c}
	6	1.00(1.23)	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{c}
Thiamethoxam 25 % WG	50 g a.i ha^{-1}	1.00(1.23)	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.00(0.71)^{c}
Neem oil	2	0.67(0.99)	0.33(0.88)^{b}	0.00(0.71)^{b}	0.00(0.71)^{b}	0.66(0.99)^{b}
Control	Nil	1.00(1.09)	1.67(1.44)^{a}	1.67(1.44)^{a}	2.00(1.56)^{a}	2.00(1.56)^{a}
CD (0.05)		NS	(0.29)	(0.65)	(0.31)	(0.34)

*Mean of 3 replications comprising 3 plants each
(Values in the parenthesis are square root transformed)
DAT: Days after treatment

Table 7. Effect of oil-based formulation of *A. paniculata* on syrphids in cowpea

Treatments	Dose (%)	Precount	Mean population (Number plant^{-1}) * Days after treatment			
			1	3	5	7
Plant extract of *A. paniculata* (70 %) + neem oil (20 %) + Triton X-100 (10 %)	1	0.67(1.05)	0.67(1.05)	0.67(1.05)	1.00(1.23)^{a}	1.00(1.23)^{a}
	2	1.00(1.17)	0.67(1.05)	0.33(0.88)	0.33(0.88)^{bc}	0.33(0.88)^{b}
	3	1.00(1.17)	0.00(0.71)	0.00(0.71)	0.00(0.71)^{c}	0.00(0.71)^{b}
	5	1.00(1.17)	0.00(0.71)	0.00(0.71)	0.00(0.71)^{c}	0.00(0.71)^{b}
	6	1.00(1.17)	0.33(0.88)	0.00(0.71)	0.00(0.71)^{c}	0.00(0.71)^{b}
Plant extract of *A. paniculata* (70 %) + pongamia oil (20 %) + Triton X-100 (10 %)	1	0.33(0.88)	0.33(0.88)	0.33(0.88)	0.67(1.05)^{ab}	1.00(1.23)^{a}
	2	0.33(0.88)	0.33(0.88)	0.33(0.88)	0.33(0.88)^{bc}	0.33(0.88)^{b}
	3	1.00(1.17)	0.67(1.05)	0.00(0.71)	0.00(0.71)^{c}	0.00(0.71)^{b}
	5	1.00(1.23)	0.00(0.71)	0.00(0.71)	0.00(0.71)^{c}	0.00(0.71)^{b}
	6	1.33(1.34)	0.00(0.71)	0.00(0.71)	0.00(0.71)^{c}	0.00(0.71)^{b}
Thiamethoxam 25 % WG	50 g a.i ha^{-1}	2.00(1.56)	0.00(0.71)	0.00(0.71)	0.00(0.71)^{c}	0.00(0.71)^{b}
Neem oil	2	1.00(1.17)	0.67(1.05)	0.00(0.71)	0.00(0.71)^{c}	0.33(0.88)^{b}
Control	Nil	0.67(1.05)	0.67(1.05)	0.67(1.05)	1.00(1.23)^{a}	1.00(1.23)^{a}
CD (0.05)		NS	NS	NS	(0.24)	(0.24)

*Mean of 3 replications comprising 3 plants each
(Values in the parenthesis are square root transformed)
DAT: Days after treatment

a) *Coccinella transversalis*

b) *Cheilomenes sexmaculata*

c) Syrphid egg

d) Syrphid adult

Plate 6: Natural enemies

4.2.3 Biometric Observations of Cowpea Plants Sprayed with Oil Based formulations of *A. paniculata.*

The influence of formulations on number of pods, number of seeds per pod, pod length and pod weight per plant is presented in Table 9.

4.2.3.1 Number of pods

Thiamethoxam 25% WG recorded highest pod number (8). This was found to be on par with 3% concentration of pongamia based formulation. Rest of the treatments were found to be on par with each other. The lowest pod number was recorded in untreated plants (4).

4.2.3.2 Number of seeds per pod

There was no significant difference in the number of seeds per pod and hence seed count obtained was not influenced by treatments.

4.2.3.3 Pod length

There was significant difference in the pod length. Among different treatments, thiamethoxam 25% WG showed superiority in length of pods (39.53 cm). This was followed by 5 and 6 % concentration of pongamia based formulation (35.97 cm and 35.70 cm respectively). This was found to be on par with 3 and 2% concentration of neem-based formulation (34.67 cm and 34.03 cm respectively). Rest of the treatments showed no significant difference. Lowest pod length was observed for untreated check (27.80 cm)

4.2.3.4 Pod weight

The pod weight obtained from a single harvest during the crop period is presented. Highest pod weight (55g $plant^{-1}$) was obtained from the plants treated with thiamethoxam 25% WG which was found to be on par with 5% concentration of pongamia based formulation (49.16 g $plant^{-1}$). Pod weight did not vary significantly for rest of the treatments. Untreated plants recorded lowest pod weight of 29.67 g $plant^{-1}$.

Table 8. Pod characteristics as influenced by oil-based formulations of *A. paniculata*.

Treatments	Dose (%)	*Number of pods	*Length of pod (cm)	*Number of seeds/pods at time of observation	**Pod weight (g/plant)
P E A (70 %) + neem oil (20 %) + T (10 %)	1	5.00[def]	31.57[cd]	12.00	42.97[bcd]
	2	5.33[cde]	34.03[bc]	11.00	34.77[cde]
	3	4.33[ef]	34.67[bc]	12.00	32.36[de]
	5	4.33[ef]	32.93[bcd]	11.67	44.67[abc]
	6	6.00[cd]	33.80[bcd]	9.00	36.36[cde]
P E A (70 %) + pongamia oil (20 %) + T (10 %)	1	5.33[cde]	32.80[bcd]	10.00	43.33[abcd]
	2	5.00[def]	32.87[bcd]	13.33	38.96[bcde]
	3	7.33[ab]	31.63[cd]	10.33	39.77[bcde]
	5	5.00[def]	35.97[b]	12.00	49.16[ab]
	6	6.33[bc]	35.70[b]	10.00	42.67[bcd]
Thiamethoxam 25 % WG	50 g a.i ha-1	8.00[a]	39.53[a]	10.33	55.50[a]
Neem oil	2	4.67[ef]	30.50[de]	11.00	41.76[bcde]
Control	Nil	4.00[f]	27.80[e]	9.33	29.67[e]
CD (0.05)		(1.17)	(3.49)	NS	(12.27)

*Mean of 3 replications comprising 3 plants DAT: Days after treatment PEA: Plant extract of *A. paniculata* T: Triton X-100
** Weight of pods obtained from single harvest

4.2.4 Phytotoxicity

Phytotoxic effects of different concentrations of oil-based formulations of *A. paniculata* at 1, 2, 3, 5, 10 and 20 % concentrations on cowpea were evaluated and observations for symptoms like yellowing, scorching, necrosis, epinasty and hyponasty were done. Results revealed that oil-based formulations of *A. paniculata* does not exhibit any phytotoxic response on cowpea plants up to a concentration of 20%.

Plate 7: Phytotoxicity study arrangement

Discussion

5. DISCUSSION

Unsystematic use of chemical insecticides along with monoculture and global climate change have emanate in major shift of pests from leaf or fruit eating caterpillars to sucking pests in many cropping ecosystems. In cowpea, sucking pests are often a barrier for quality production. Over 98% of insecticides sprayed reach non target species due to wind drifts. Meanwhile run off also carries insecticides into aquatic environments, human settlements, grazing lands and other undeveloped areas, potentially upsetting other species. Over time, continuous application of pesticides increases pest resistance, while its adverse effects on other species facilitates pest resurgence.

This demands the development of novel botanical formulations which are environmentally approachable and economically feasible. Botanical pesticides are usually less effective when pest build up is very high in field conditions. Biopesticide formulations comprising more than one plant extracts may give synergistic effect to the finished product. Among diverse formulations, emulsifiable concentrates (EC) are more ideal for farmers because of high biological activity, good storage stability and easiness in handling (Alan, 2008; Vanitha, 2010; Prajapati *et al*., 2014).

In this background, the current investigation was executed as a preliminary step to advance an eco-friendly bio pesticide formulation using extract of *Andrographis paniculate* and oils like neem oil and pongamia oil for confronting sucking pests of cowpea.

5.1 IN VITRO EVALUATION OF DIFFERENT FORMULTION OF *A. paniculata*

The present investigation was emphasized on the advancement of oil-based formulations of *A. paniculata.* Different concentration (1, 2, 3 and 5%) formulation of *A. paniculata* were evaluated against *A. craccivora* and it was found that 1% neem oil-based formulation was significantly superior with 75.00 per cent mortality at 1 DAT. Accordingly neem oil-based formulation exhibited 95.00 percent mortality at 3 DAT and 100.00 percent mortality at 5 and 7 DAT. This was found in conformity with study of Lowery and Isman (1994) who found that neem seed oil (NSO) when applied to leaf at a concentration of 1% resulted in 94.00 to 100.00 percent mortality of second instar nymphs of green peach aphid, *M. persicae* and lettuce aphid, *Nasonoviaribis nigri* (Mosley). Srivastava and Parmer (1985) reported that 1% emulsion spray of neem oil applied to sorghum killed all the aphids in 1-2 hours. Treatment at 0.5% was equally promising and treatment at 0.2 and 0.1% took about 24 and 48 h, respectively, to obtain the same results and also 0.2% emulsion prevented the build of *M. sacchari* in field for up to 3 weeks.

Aphids when treated with 2% concentration of neem oil-based formulation was superior with 91.67 percent mortality after 1 DAT and recorded 100.00 percent mortality on 3 DAT. This result is in agreement with study of Kraiss and Cullan (2008) who reported that both neem seed oil and azadirachtin significantly increased nymphal mortality of *Aphis glycines* about 77.00 and 80.00 percentage respectively. Similar study was conducted by Stark and Rangus (1994) to find out effect of 'Margosan- O' (MO)on the pea aphid, *Acyrthosiphon pisum* (Harris). Results revealed that MO significantly decreased population build-up of aphids in a concentration dependent approach. At a concentration of 100.00 mg litre^{-1} of azadirachtin, population increase was 3.5 times lower than untreated check.

Upon treatment with 3% concentration of neem oil-based formulation showed best results and aphids recorded 98.33 and 100.00 percent mortality on 1st and 3rd DAT respectively. When increasing the concentration to 5% formulation, 100.00 percent mortality was obtained within 1 DAT. This was found to be in line with study of Biswas (2013) where neem leaf extracts at a concentration of 50g neem seed per litre of water reduced 63.16-72.55% aphid population in mustard. Lawrey *et.al*., (1993) reported that neem seed oil and extract reduced aphid numbers up to 50 % at a concentration range of 0.2 to 1.4 % under field conditions and were found to be equally effective as pyrethrum for aphid control in pepper and strawberry.

Tanq *et.al*., (2002) reported neem oil spray over aphids drastically reduces adult and nymph longevity, moulting of nymphs and adult fecundity at all concentrations. As per finding of Shannag *et al*., (2014) aphids fed with leaves containing neem-based insecticides, the honeydew excretion rate was significantly decreased, to 14-40% of the control, thus demonstrating feeding deterrence. Insecticidal property of neem oil may be credited to active principle in neem namely azadirachtin, the tetranortriterpenoid limonoid (Kumar, 2016).

In the present study oil-based formulation of pongamia at 1% concentration was reported to be effective against *A. craccivora* causing mortality of 71.67 per cent at 1

DAT. Accordingly 2, 3 and 5 % concentrations exhibited 100.00 percent mortality at 5, 3 and 1 DAT respectively. Result was found in conformity with the study of Stepanycheva *et al.* (2014). They standardized the bio pesticide formulation containing *Sapindus saponaria* extract, pongamia oil, and Tween 20 in the ratio of 1: 8:1 at 3% concentration was effective against *M. persicae* recording 95.00 per cent mortality. Bio efficacy of combinations of plant extracts including pongamia oil was less explored and researches were more focused on the insecticidal properties of pongamia oil alone.

The higher percentage mortality reported in oil-based formulations of pongamia may be due to the pesticidal property of pongamia oil which was in agreement with the study of Kumar and Singh (2002). They concluded that flavonoids, terpenoids chalcones and steroids are responsible for insecticidal activity of pongamia oil. Moreover, the toxicity of pongamia oil is also reported against broad mite (*Polyphagotarsonemus latus*) Reddy and Kumar (2006) and Veena et al. (2017).

Toxicity of pongamia oil in *A. gossypii* was reported by Vinodhini and Malaikozhundan (2011) and thrips (*Scirtothrips dorsalis*) by Meena and Tayde (2017) at 1- 4 % concentration.

In the current research work, combination of *A. paniculata* extract and oils expressed synergistic effect against aphids compared to plant extract and oils alone. The synergistic effect of neem oil was supported by Mohan *et.al*., (2007) who's study revealed that combined application of neem oil with *Beuvaria bassiana* was found to have synergistic effect on insect mortality. Study conducted by Radhika and Sahayaraj (2014) concluded that either neem oil or pongam oil can be blended with monocrotophos for the management of *Spodoptera litura* larvae.

The synergistic effect of pongamia oil was well supported by the findings of Kumar and Singh (2002). They found that oil-based formulation of pongamia have good emulsion stability and excellent synergistic effect with other botanicals.

Among botanicals, 2% aqueous *A. paniculata* extract accounted for maximum reduction in population of aphids, leaf miners, thrips and defoliators infesting brinjal. and it also conserved natural enemies like coccinellids (Suganthy and Sakthivel 2012; Suganthy *et.al.*, 2015). The insecticidal properties of *A. paniculata* is accounted for the presence of a diterpenoid lactones (andrographolides), paniculides, flavonoids and farnesols (Ramya *et al*., 2011).

Andrographolide overturn the feeding of the female rice leaf hopper at concentrations of as low as 1.00 ppm. By root-immersion method, the lowest concentration of extract that suppressed feeding was 1, 600 ppm. The antifeedant activity of andrographolide was similar to the feeding-deterrent activities bensultap and cartap (Widiarta *et.al*., 1997). The insecticidal activity of *A. paniculate* detected in present study can be validated by other findings of Singh *et al.* (2014); Madihah *et al*. (2018) and Prema *et al*. (2018);

In general, mode of action of oils is not yet confirmed. Oils are known to induce mortality by suffocation as per findings of Don-Pedro (1989). They can also act as insect growth regulators (IRGs) by affecting insect metamorphosis and antifeedants (Weaver and Subramanyam, 2000). For further ratification regarding the relative efficacy of formulations (neem and pongamia based formulations), the two selected formulations were screened in the field.

5.2 EVALUATION OF EFFECTIVE FORMULATION FOR FIXING THE DOSE

Field evaluation is an eminent methodology for credible substantiation of the findings obtained from laboratory experiments. The promising outcomes attained under invitro condition may not replicate in field situations due to numerous abiotic and biotic stress existing in open field conditions. Hence forth promising treatments selected from laboratory studies was tested under field condition to compare their field efficacy. Field evaluation was conducted using the effective formulations *viz.*, neem and pongamia oil-based formulations along with neem oil 2% and thiamethoxam 25% WG as check.

Mosaic resistant cowpea variety Geethika with long thick green fleshy pods and reddish-brown seeds with an average yield of 27.6t/ha was selected for pot culture experiments. A consistent population of pests was maintained in the field by avoiding plant protection interventions. After recording the precount of pests, treatments were applied at the vegetative phase of the crop i.e., 30 days after planting. 1, 2, 3, 5 and 6% concentration of neem and pongamia based formulation of *A. paniculata* were tested against *A. craccivora*.

At 1 DAT, mean population of aphid was found to be zero in 6% concentration of both neem oil and pongamia oil-based formulations of *A. paniculata*. This was statistically similar with 3 and 5% concentration of neem oil-based formulation as well as 5% concentration of pongamia based formulations. At 3 DAT, maximum reduction in pest population (0.00 per plant) over untreated control was recorded for 3 and 5% concentration of neem oil-based formulations of *A. paniculata*. At 5 DAT, 2% concentration of neem oil-based formulations and 3 and 5 % concentration of pongamia based formulations exhibited 100 % mortality of pest. At 7 DAT, 2% concentration of pongamia based formulation of *A. paniculata* recorded zero population of aphids and plants treated with 2% neem oil (check) recorded re-establishment of cowpea aphids.

Maximum reduction in aphid population on cowpea was conveyed by neem oil-based formulation of *A. paniculata*. Higher efficacy of neem oil against sucking pests was in harmony with the studies of Mansour *et al*., (1993); Mansour *et al*., (1997) and Sakthivel *et al*., (2012) and Rani and Shivaraman (2019).

Hence it can be concluded that neem oil-based formulation at 6% concentration or above was found to be effective in managing sucking pests of cowpea and was equally effective as chemical insecticides thiamethoxam 25% WG when applied at fortnightly intervals. Literature pertaining to progress of oil-based formulations of *A. paniculata* is scanty. Bernice (2000) reported that leaf extract of *A. paniculata* in combination with neem oil emulsion 2.5% and garlic at 2% possess deterrent effects on aphids and epilachna beetle.

5.2.1Safety Evaluation of Oil based formulations of *A. paniculata* on Natural Enemies and ants associating with aphids in Cowpea Ecosystem.

After treatment applications, maximum population of natural enemies including coccinellid beetles and syrphids were found in control plot and minimum in 3, 5 and 6% concentration of neem based formulations, 5 and 6% concentration of pongamia based formulations and chemical check on 1 DAT. Re-establishment of natural enemies were observed for Neem oil+ Triton-X-100 after 7 DAT. Results are corelated with findings of Feldhege and Schmutterer (1993). They reported that 20ppm concentration of azadiractin led to significant reduction in fitness of *Encarsia formosa*. Result was in agreement with findings of Osman and Bradley (1993) with reports of negative effects of neem oil on parasitoid *Apanteles glomeratus* population. Findings was again validated by studies of Raghuraman and Singh (1998) reporting neem oil at 0.3, 0.6, 1, 2, 2.5 and 5% concentration led to feeding and ovipositional deterrence, sterility, toxicity and insect growth regulatory effects on *Trichogramma chilonis*. Similar findings were also made by Raguraman and Singh (1999) and Rao *et al*., (2007)

5.2.2 Biometric Observations of Cowpea PlantsSprayed with Oil Based formulations of *A. paniculata*

In the present study, there was no significant difference observed between treatments in biometric parameters viz., number of pods, pod length, number of seeds per pod and pod weight. A drop in the overall pod weight might be due to the presence of very high population of the sucking pests in the initial crop phase that have delayed the plants from expressing the outcome of treatments in the later phase of the crop. Vichitbandha and Chandrapatya (2011) reported that damage more than 50 per cent caused by sucking pests resulted in retarded growth and yield loss in crops.

5.2.3 Phytotoxicity studies

Oil-based formulations of *A. paniculata* do not conform any form of phytotoxic effect on cowpea plant for a concentration ranging from 1- 20%. At present no previous reports are found to validate the fin

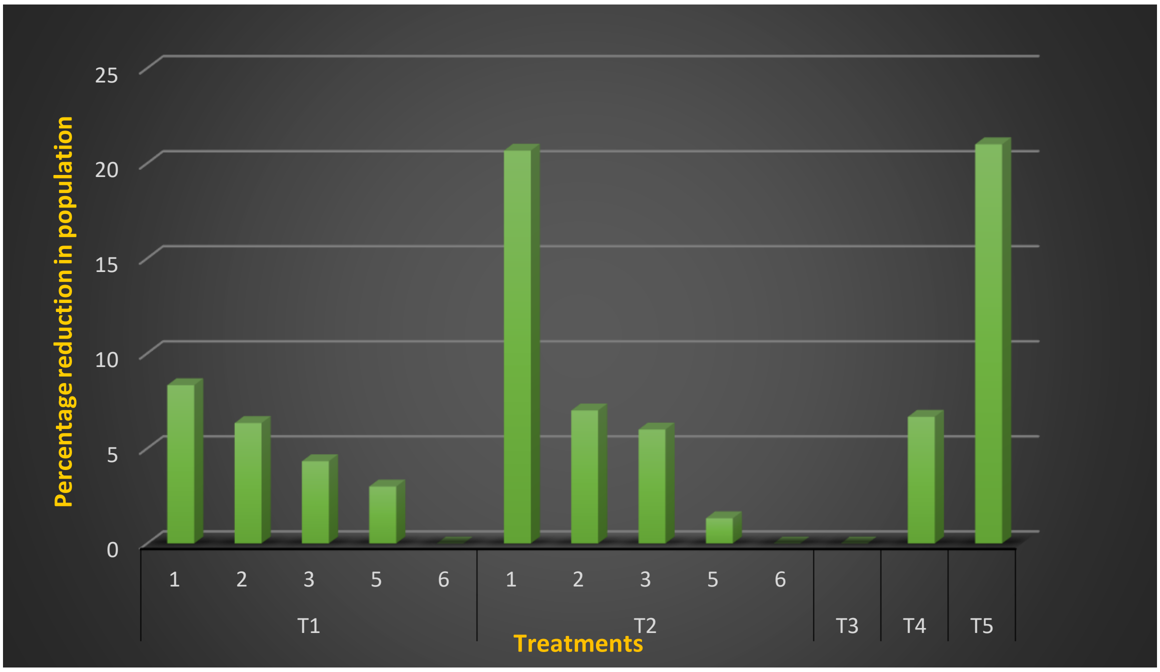


Fig. 1: Per cent reduction in the population of cowpea aphid at 1 DAS

T1 - Plant extract of *A. paniculata* (70 %) + neem oil (20 %) + Triton X-100 (10 %)

T2 - Plant extract of *A. paniculata* (70 %) + pongam oil (20 %) + Triton X-100 (10 %)

T3 - Thiamethoxam 25 % WG

T4 – Neem oil 2%

T5 - Control

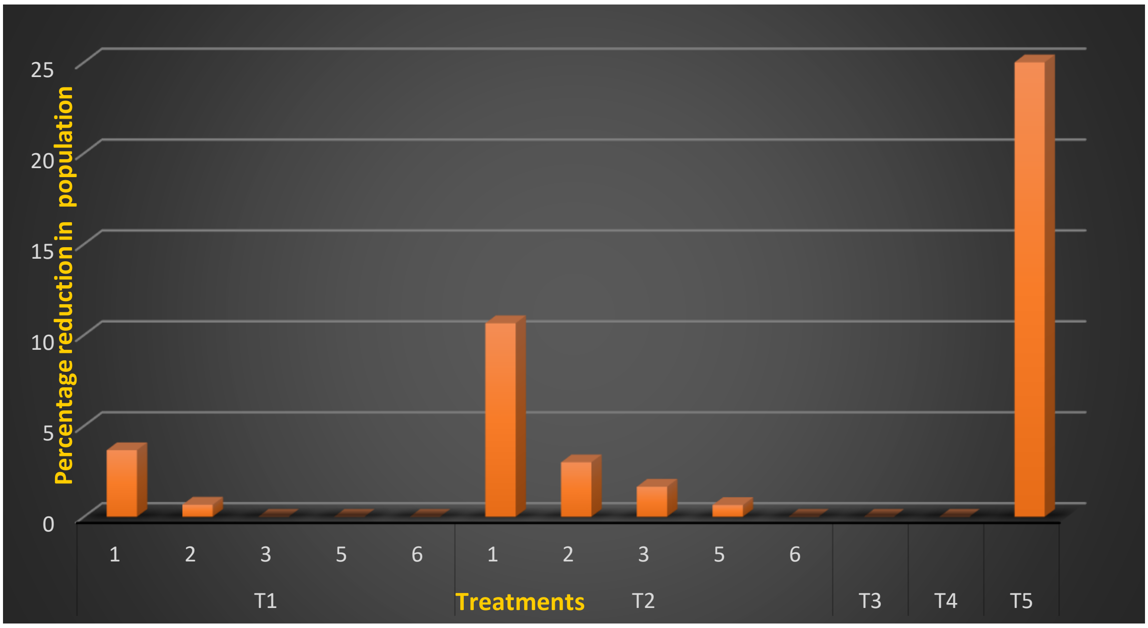


Fig. 2: Per cent reduction in the population of cowpea aphid at 3 DAS

T1 - Plant extract of *A. paniculata* (70 %) + neem oil (20 %) + Triton X-100 (10 %)

T2 - Plant extract of *A. paniculata* (70 %) + pongam oil (20 %) + Triton X-100 (10 %)

T3 - Thiamethoxam 25 % WG

T4 – Neem oil 2%

T5 – Control

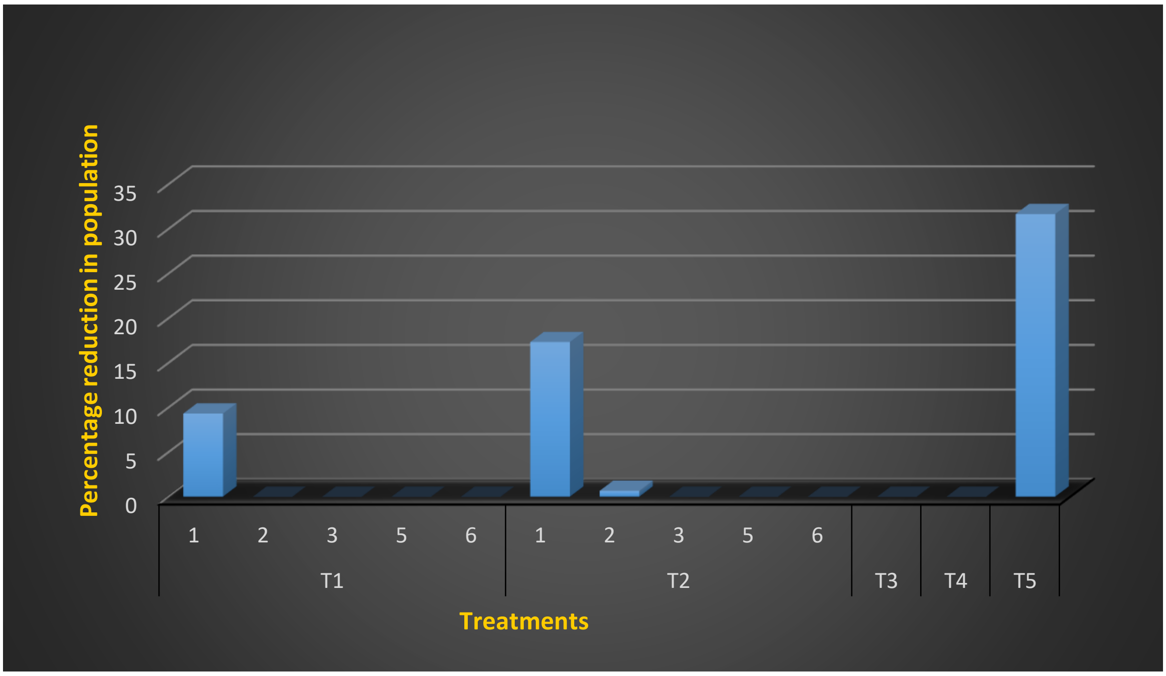


Fig. 3: Per cent reduction in the population of cowpea aphid at 5 DAS

T1 - Plant extract of *A. paniculata* (70 %) + neem oil (20 %) + Triton X-100 (10 %)

T2 - Plant extract of *A. paniculata* (70 %) + pongam oil (20 %) + Triton X-100 (10 %)

T3 - Thiamethoxam 25 % WG

T4 – Neem oil 2%

T5 - Control

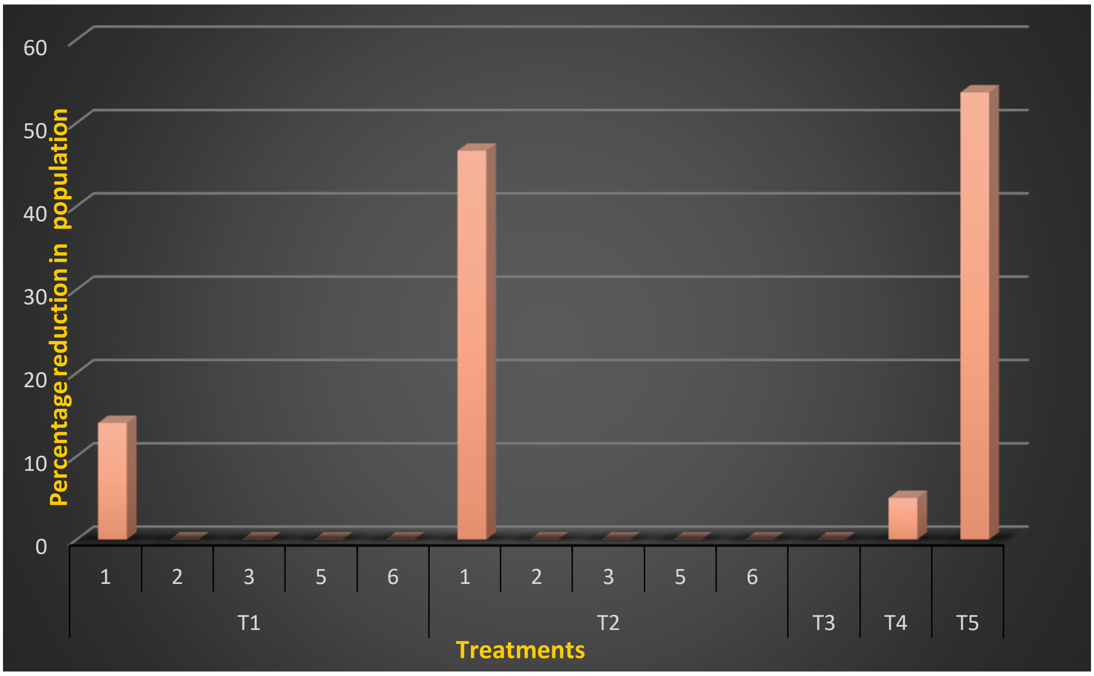


Fig. 4: Per cent reduction in the population of cowpea aphid at 7 DAS

T1 - Plant extract of *A. paniculata* (70 %) + neem oil (20 %) + Triton X-100 (10 %)

T2 - Plant extract of *A. paniculata* (70 %) + pongam oil (20 %) + Triton X-100 (10 %)

T3 - Thiamethoxam 25 % WG

T4 – Neem oil 2%

T5 - Control

Summary

6. SUMMARY

Cowpea, *Vigna unguiculata* (L.) Walp. is an important food legume and an integral part of traditional cropping systems in the semi-arid regions of the tropics. Over the years, insect pests attack has been found as one of the major biotic constraints of vegetable production across the country. Among the arthropods infesting cowpea, aphids, caterpillars, and pod bugs accounted for high levels of infestations, persistence and overall damage inflicted on the crop. Annual yield loss due to the insect pests has been estimated about 30 per cent and complete crop failure may occur especially in situation where control measures are not applied.

The use of synthetic insecticides has been the most widely used control measure and its uses has led to numerous threats such as residue problems, environmental pollution, pest resurgence and secondary pest outbreak. This has necessitated the search for alternative control measures that are eco-friendly and pose no health and environmental threat. Plants are well known to have a range of secondary metabolites that are indispensable for their growth and development and are absolutely necessary in protection against insect pests and pathogens. Plant originated secondary metabolites plays a chief role in curtailing insect damage on crops. The use of plants in insect pest management is not only useful for suppression of pest population, but also helps to maintain the sound ecological balance. *A. paniculata* is a bitter annual herb claimed to possess active ingredients of insecticidal value. However botanical pesticides were found less effective when the pest incidence is very high in the field conditions. Thus, researches are more focused on development of formulations involving more than one plant extract, which often holds more insecticidal potential due to synergistic action.

With this backdrop, the present investigation entitled "Oil based biopesticide from *A. paniculata* (Burm.f.) Nees against sucking pests of cowpea" was conducted during period 2018 -2020, in Department of Agricultural Entomology, College of Agriculture, Vellayani with an objective to develop oil based ready to use biopesticide from *A. paniculata* against important sucking pests in cowpea and to fix the optimum dose for managing the pests.

Study comprised of *invitro* and field evaluation of oil-based formulations. Accordingly, during the laboratory evaluation, mortality of aphids on 1 DAT due to the oil-based formulations of neem at 1, 3 and 5% concentration was 75.00, 95.00 and 100.00% respectively. At 2% concentration of neem-based formulation, 100% mortality was obtained within 24 hours and for pongamia based formulation 100% mortality achieved was achieved at 5 DAT . At 7 DAT, extract of *A. paniculata* + Triton -X-100 exhibited 100% mortality. At 3% concentration, neem-based formulation resulted 100% mortality within 24 hours and pongamia based formulation took 72 hours to reach 100% mortality. Upon 5 DAT, extract of *A. paniculata* + Triton -X-100 and pongamia oil + Triton -X-100 exhibited 100% mortality. At 5% concentration of formulations, both neem and pongamia based formulations of *A. paniculata* exhibited 100 % mortality within 3 DAT and rest of treatments also recorded cent percentage mortality.

Results of the laboratory experiment revealed that neem oil and pongamia oil-based formulations of *A. paniculata* at 5% concentration was sufficient for 100 per cent mortality of aphids at 24 hours after treatment (HAT).

Field evaluation was carried out using cowpea variety Geethika with the two selected promising treatments (neem and pongamia based formulation of *A. paniculata*) to assess the field efficacy against sucking pests. Consistent population of pest was maintained in the field by avoiding plant protection measures. 1, 2, 3, 5 and 6% concentrations of the above selected treatments were evaluated with thiamethoxam 25% WG and neem oil 2% as check. One spray was given at 30 DAT i.e., during the vegetative phase. At 1 DAT, lowest population of cowpea aphids was recorded at 6% concentration of both neem oil based and pongamia oil-based formulations. This was statistically on par with 3 and 5% concentration of neem-based formulation as well as 5% concentration of pongamia based formulation. At 3 DAT, no mortality of aphids were observed at 3 and 5 % concentrations of neem oil-based formulation recorded least population. At 5 DAT, 2% concentration of neem-based formulation and 3 and 5 % concentration of pongamia based formulation recorded zero population of aphids. Upon 7 day after treatment, plants treated with 2% concentration of pongamia based formulation also recorded zero population of aphids.

Results of the field experiment revealed that neem oil and pongamia oil-based formulations of *A. paniculata* at 6% concentration was sufficient for 100 per cent mortality of aphids at 24 hours after treatment (HAT).

Population of natural enemies including syrphids and coccinellid beetles were recorded in all treatments compared to neem oil 2% and thiamethoxam 25% WG at 1, 3, 5 and 7 days after treatments application. Maximum population was recorded in control plot and minimum in chemical check.

The highest pod weight of 49.16 g plant $^{-1}$ was obtained at 5% concentration of pongamia based formulation but treatments did not vary significantly. All treated plants exhibited improvement in growth attributes like pod numbers, pod length and pod weight as against untreated control. Phytotoxicity studies using treatments like plant extract of *A. paniculata* + neem oil + Triton X-100, plant extract of *A. paniculata* + pongamia oil + Triton X-100 and plant extract of *A. paniculata* + Triton X-100 at 1, 2, 3, 5, 10 and 20 % concentration were carried out and observations for symptoms including yellowing, scorching, necrosis, epinasty and hyponasty were made and graded according to CIBRC (Central Insecticides Board and Registration Committee) protocol. Results revealed that oil-based formulations of *A. paniculata* exhibited no phytotoxic response up to a concentration of 20%.

The salient findings of the investigation are:

- Oil based formulations of *A. paniculata* + neem oil +Triton-X-100 and *A. paniculata* + pongam oil +Triton-X-100 at 6% concentration records 100% mortality of cowpea aphid and was on par with 3 and 5% concentration of *A. paniculata* + neem oil +Triton-X-100 and 5% concentration of *A. paniculata* + pongam oil +Triton-X-100.

- Oil based formulations of *A. paniculata*+ neem oil +Triton-X-100 at concentration of 3% or above records 100 % mortality within 3 DAT. 3% and above concentration of *A. paniculata*+ pongam oil +Triton-X-100 records 100% mortality 5 DAT.

- Plants treated with Neem oil + Triton-X-100 (2%) expressed good mortality rates during initial days of spray but reoccurrence of the pest was noticed 7 DAT.

- Maximum population of natural enemies was found in control plot and minimum in chemical check. Re-establishment of natural enemies was observed for Neem oil+ Triton-X-100 after 7 DAT.

- No phytotoxic response of oil-based formulations of *A. paniculata* on cowpea was observed up to a concentration of 20.
- Cowpea pods in plots treated with chemical check was superior in length and weight and quantity.
- For ecofriendly management of sucking pests of cowpea, efficacy of oil-based formulations comprising *A. paniculata* + neem oil+ Triton-X-100 (7:2:1) at a concentration of 6% was on par with the chemical treatments when applied at fortnightly intervals.
- The oil based biopesticides which are efficient in managing these pests should be made commercially available to farmers which helps them to control these pests in an organic way.

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Oil based biopesticide from *Andrographis paniculata* (Burm.f.) Nees against sucking pests of cowpea

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ABSTRACT

The study on “Oil based biopesticide from *Andrographis paniculata* (Burm.f.) Nees against sucking pests of cowpea” was conducted at College of Agriculture, Vellayani during 2018 to 2020 with an objective to develop oil based ready to use formulations against sucking pests of cowpea and to evaluate the formulation for its efficacy. In vitro screening of different oil-based formulations of *A. paniculate* with EOS ratio of 7:2:1 was done using *Aphis craccivora* as test insect. 1, 2, 3 and 5% concentration of formulations comprising plant extract of *A. paniculata* +neem oil + Triton -X-100, plant extract of *A. paniculata* +pongamia oil+ Triton-X-100, plant extract of *A. paniculata* + Triton -X-100, Neem oil+ Triton -X-100 + Water (2:1:7)(check) and Pongamia+Triton -X-100 + Water (2:1:7) (check) were sprayed directly over aphids and observation for percentage mortality were recorded at 1, 3, 5 and 7 days after spraying.

Results of the laboratory experiment revealed, oil-based formulations of neem at 1% concentration recorded 75.00% mortality of aphidsat1 day after treatment (DAT). Mortality at 3 DAT was 95.00%and 5 and 7 DAT were 100.00%. At 2% concentration of formulations, neem-based formulation exhibited 100% mortality within 24 hours and pongamia based formulation exhibited 100.00% mortality within 5 days after treatment application. At 7 DAT, plant extract of *A. paniculata* + Triton -X-100 exhibited 100.00% mortality. At 3% concentration of oil-based formulations, neem-based formulation exhibited 100.00% mortality within 24 hours and pongamia based formulation within 72 hours. Plant extract of *A. paniculata* + Triton -X-100 and pongamia oil + Triton -X-100 exhibited 100.00% mortality at 5 DAT. At 5% concentration of formulations, both neem and pongamia based formulations of *A. paniculata* exhibited 100.00% mortality within 3 day after treatment and rest of treatments also recorded cent percentage mortality.

A pot culture experiment was carried out in cowpea variety Geethika with two superior treatments to assess the field efficacy against sucking pests. 1, 2, 3, 5 and 6% concentrations of the selected treatments were evaluated with neem oil 2% and thiamethoxam 25% WG as checks. Pre count of pest and natural enemies were documented and a single round of spraying was undertaken at the vegetative phase of crop i.e., 30 DAP. In field evaluation, at 1 DAT, least population of aphids were recorded at 6% concentration of extract of *A. paniculata* + neem oil + Triton X- 100 and extract of

A. paniculata + pongamia oil + Triton X- 100 sprayed cowpea plants and the results were statistically on par with 5% concentration of extract of *A. paniculata* + pongamia oil + Triton X-100, 3 and 5% concentration of *A. paniculata* + neem oil + Triton X-100 and chemical check thiamethoxam 25% WG. Neem oil-based formulation at 3 and 5% concentration recorded least population (0.00 per plant) at 3 DAT. No aphids were observed in plants treated with neem-based formulation (2%) and pongamia based formulation at 3 and 5% on 5^{th} day of treatment. Plants treated with 2% concentration of pongamia based formulation recorded zero population of aphids at 7 DAT while recurrence of aphids was observed in plants treated with neem oil (2%).

Post count of natural enemies viz. syrphids and coccinellid beetles were recorded in all treatments and compared with botanical and chemical check at interval of 1, 3, 5 and 7 DAT. Control plot recorded maximum and chemical check recorded minimum population of natural enemies.

On evaluating the biometric characters, highest pod weight of 49.16 g $plant^{-1}$ was obtained in 5% concentration of pongamia based formulation. Phytotoxicity studies using treatments like plant extract of *A. paniculata* + neem oil + Triton X-100, plant extract of *A. paniculata* + pongamia oil + Triton X-100 and plant extract of *A. paniculata* + Triton X-100 at 1, 2, 3, 5, 10 and 20 % concentration were carried out and observations for symptoms including yellowing, scorching, necrosis, epinasty and hyponasty were made and graded according to CIBRC (Central Insecticides Board and Registration Committee) protocol. Results revealed that oil-based formulations of *A. paniculate* does not produce any kind of phytotoxic response on cowpea within a range of 1- 20% concentration.

From the above findings it was concluded that for ecofriendly management of sucking pests of cowpea, efficacy of oil-based formulations comprising *Andrographis paniculata* + neem oil+ Triton-X-100 (7:2:1) at a concentration of 6% was on par with the chemical treatments when applied at fortnightly intervals.

സംഗ്രഹം

കിരിയാത്തിൽ (ആൻഡ്രോഗ്രാഫിസ് പാനിക്യുലാറ്റ) നിന്നും പയറിലെ നീരൂറ്റി കുടിക്കുന്ന കീടങ്ങൾക്ക് എതിരെ എണ്ണ അധിഷ്ഠിത ജൈവകീടനാശിനി ഉത്പാദിപ്പിക്കുന്നതിനും അതിൻറെ ഫലപ്രാപ്തി വിലയിരുത്തുന്നതിനുമായി 2018 - 2020 കാലയളവിൽ വെള്ളായണി, കാർഷിക കോളേജിൽ വെച്ച് എം. എസ്. സി. എൻടൊമോളജി വിദ്യാർതി നടത്തിയ ഗവേഷണ പഠനങ്ങളുടെ ഫലങ്ങളാണ് ചുവടെ ചേർക്കുന്നത്.

കിരിയാത്ത് സത്ത്, എണ്ണ, ട്രൈറ്റൺ-എക്സ്-100 എന്നീ പദാർത്ഥങ്ങൾ 7:2:1 എന്ന അനുപാതത്തിൽ ചേർത്ത് വിവിധ എണ്ണ അധിഷ്ഠിത ഫോർമുലേഷനുകൾ തയ്യാറാക്കി, പയർ മുഞ്ഞയെ (അഫിസ് ക്രാസിവോറ) പരീക്ഷണ പ്രാണിയായി ഉപയോഗിച്ച് ഇൻ വിട്രോ സ്ക്രീനിംഗ് നടത്തി. കിരിയാത്ത് സത്ത് + വേപ്പെണ്ണ + ട്രൈറ്റൺ-എക്സ്-100, കിരിയാത്ത് സത്ത് + പൊങ്കാമിയ എണ്ണ + ട്രൈറ്റൺ-എക്സ്-100, കിരിയാത്ത് സത്ത് + ട്രൈറ്റൺ-എക്സ്-100, വേപ്പെണ്ണ + ട്രൈറ്റൺ-എക്സ്-100 + വെള്ളം (2:1:7) (ചെക്ക്), പൊങ്കാമിയ എണ്ണ +ട്രൈറ്റൺ-എക്സ്-100 + വെള്ളം (2:1:7) (ചെക്ക്) എന്നീ മിശ്രിതങ്ങളുടെ ഒന്നും രണ്ടും മൂന്നും അഞ്ചും ശതമാനം വീര്യമുള്ള ഫോർമുലേഷനുകൾ കീടത്തിനു മേൽ നേരിട്ട് തളിച്ച ശേഷം 1, 3, 5, 7 എന്നീ ദിവസങ്ങളിൽ അവയുടെ മരണനിരക്ക് നിരീക്ഷിച്ചു.

ലബോറട്ടറി പരീക്ഷണത്തിന്റെ ഫലങ്ങൾ നിരീക്ഷച്ചപ്പോൾ 1 ശതമാനം വീര്യമുള്ള കിരിയാത്തിന്റെ വിവിധ എണ്ണ അധിഷ്ഠിത ഫോർമുലേഷന്റ പ്രയോഗത്തിന് ശേഷം ഒന്നാം ദിവസം 75.00% മരണനിരക്ക് രേഖപ്പെടുത്തി. മൂന്നാം ദിവസം മരണനിരക്ക് 95.00% വും, അഞ്ചും, ഏഴും ദിവസങ്ങളിൽ മരണനിരക്ക് 100.00% വും രേഖപ്പെടുത്തി. 2 ശതമാനം വീര്യമുള്ള വേപ്പെണ്ണ അധിഷ്ഠിത കിരിയാത്ത് ഫോർമുലേഷൻ, 24 മണിക്കൂറിനുള്ളിലും, 2 ശതമാനം വീര്യമുള്ള പൊങ്കാമിയ എണ്ണ അധിഷ്ഠിത കിരിയാത്ത് ഫോർമുലേഷൻ അഞ്ചു ദിവസത്തിനുള്ളിലും 100.00% മരണനിരക്ക് രേഖപ്പെടുത്തി.

ഏഴാം ദിവസത്തിൽ, കിരിയാത്ത് സത്ത് + ട്രൈറ്റൺ-എക്സ്-100 100.00% മരണനിരക്ക് രേഖപ്പെടുത്തി. ഫോർമുലേഷനുകൾ 3 ശതമാനം വീര്യത്തിൽ ഉപയോഗിച്ചപ്പോൾ, വേപ്പ് അധിഷ്ഠിത ഫോർമുലേഷൻ 24 മണിക്കൂറിനുള്ളിലും പൊങ്കാമിയ അധിഷ്ഠിത ഫോർമുലേഷൻ 72 മണിക്കൂറിനുള്ളിലും 100.00% മരണ നിരക്ക് രേഖപ്പെടുത്തി. കിരിയാത്ത് സത്ത്+ ട്രൈറ്റൺ-എക്സ്-100, പൊങ്കാമിയ എണ്ണ + ട്രൈറ്റൺ-എക്സ്-100 എന്നീ കീടനാശിനി ഉപയോഗിച്ച് 5 ദിവസത്തിന് ശേഷം 100.00% മരണനിരക്ക് രേഖപ്പെടുത്തി.

ജൈവകീടനാശിനിയുടെ ഫീൽഡിൽ ഉള്ള ഫലപ്രാപ്തി വിലയിരുത്തുന്നതിനായി ലബോറട്ടറി പരീക്ഷണങ്ങളുടെ അടിസ്ഥാനത്തിൽ രണ്ട് മികച്ച കീടനാശിനി മിശ്രിതങ്ങൾ തിരഞ്ഞെടുക്കുകയും അവയുടെ 1, 2, 3, 5, 6 ശതമാനം വീര്യമുള്ള ഫോർമുലേഷനുകളുടെ ഫലപ്രാപ്തി, വേപ്പ് എണ്ണ 2 ശതമാനം, തയാമെത്തോക്സാം 25% WG എന്നീ ചെക്കുകളുടെ ഒപ്പം താരതമ്യ പഠനം നടത്തി വിലയിരുത്തി. കീടങ്ങളുടെയും അവയുടെ മിത്രകീടങ്ങളുടെയും മുൻ എണ്ണം രേഖപ്പെടുത്തുകയും വിളയുടെ പറിച്ചു നടൽ കഴിഞ്ഞു 30 ദിവസത്തിന് ശേഷം ഒരു വട്ടം കീടനാശിനി പ്രയോഗം നടത്തുകയും ചെയ്തു. തുടർന്നുള്ള ഫീൽഡ് നിരീക്ഷണങ്ങളിൽ 6 ശതമാനം വീര്യമുള്ള കിരിയാത്ത് സത്ത് + വേപ്പ് എണ്ണ + ട്രൈറ്റൺ എക്സ്-100, കിരിയാത്ത് സത്ത് + പൊങ്കാമിയ എണ്ണ + ട്രൈറ്റൺ എക്സ്-100 എന്നീ ഫോർമുലേഷനുകൾ തളിച്ച ചെടികളിൽ മുഞ്ഞകളുടെ എണ്ണം ഏറ്റവും കുറവ് നിരീക്ഷിച്ചു. 5 ശതമാനം വീര്യമുള്ള കിരിയാത്ത് സത്ത് + പൊങ്കാമിയ എണ്ണ + ട്രൈറ്റൺ എക്സ്-100, 3, 5 ശതമാനം വീര്യമുള്ള വേപ്പ് എണ്ണ അടിസ്ഥാനമാക്കിയുള്ള ഫോർമുലേഷൻ, തയാമെത്തോക്സാം 25% WG എന്നീ പ്രയോഗങ്ങൾ തുല്യമായ പ്രവർത്തനം രേഖപ്പെടുത്തി. കീടനാശിനി പ്രയോഗത്തിന് മൂന്ന് ദിവസങ്ങൾക് ശേഷം, 3 ഉം 5 ഉം ശതമാനം വീര്യമുള്ള വേപ്പ് എണ്ണ അടിസ്ഥാനമാക്കിയുള്ള

ഫോർമുലേഷൻ ഏറ്റവും കുറവ് മുഞ്ഞകളുടെ എണ്ണം (ഒരു ചെടിക്ക് 0.00 എണ്ണം) രേഖപ്പെടുത്തി. പ്രയോഗങ്ങൾക് ശേഷം അഞ്ചാം ദിവസം 2 ശതമാനം വേപ്പ് എണ്ണ അടിസ്ഥാനമാക്കിയുള്ള ഫോർമുലേഷനും പൊങ്കാമിയ അടിസ്ഥാനമാക്കിയുള്ള ഫോർമുലേഷനും (3%, 5%) തളിച്ച പയർ ചെടിയിൽ മുഞ്ഞകളൊന്നും കണ്ടെത്തിയില്ല. 2 ശതമാനം വീര്യമുള്ള പൊങ്കാമിയ അടിസ്ഥാനമാക്കിയുള്ള ഫോർമുലേഷൻ തളിച്ച ചെടികളിൽ 7 ദിവസത്തിന് ശേഷം മുഞ്ഞകളുടെ എണ്ണം പൂജ്യമായി രേഖപ്പെടുത്തി. വേപ്പിൻ എണ്ണ (2%) തളിച്ച സസ്യങ്ങളിൽ കീടങ്ങളുടെ ആവർത്തനം കണ്ടെത്തി.

കീടനാശിനി പ്രയോഗത്തിന് ശേഷമുള്ള മിത്രകീടങ്ങളുടെ എണ്ണം. 1, 3, 5, 7 ദിവസ ഇടവേളകളിൽ പരിശോധിച്ച് അവ ജൈവ-രാസ പ്രയോഗങ്ങളുമായി താരതമ്യ പഠനം നടത്തിയപ്പോൾ ഏറ്റവും കൂടുതൽ സിർഫിഡുകളുടെയും സുന്ദരി വണ്ടുകളുടെയും എണ്ണം നിയന്ത്രണ പ്ലോട്ടുകളിൽ കണ്ടെത്തി. രാസകീടനാശിനി തളിച്ച പയർ ചെടികളിൽ ഏറ്റവും കുറവ് മിത്രകീടങ്ങളുടെ എണ്ണവും നിരീക്ഷിച്ചു.

ബയോമെട്രിക് സവിശേഷതകൾ വിലയിരുത്തുമ്പോൾ, 5 ശതമാനം പൊങ്കാമിയ അധിഷ്ഠിത ഫോർമുലേഷൻ തളിച്ച ചെടികളിൽ നിന്ന് ഏറ്റവും ഉയർന്ന പോഡ് ഭാരം ലഭിച്ചു (ഒരു ചെടിയിൽ നിന്ന് 49.16 ഗ്രാം). തുടർന്ന് ഫൈറ്റോടോക്സിസിറ്റി പഠനങ്ങൾക്കായി കിരിയാത്ത് സത്ത് + വേപ്പ് എണ്ണ + ട്രൈറ്റൺ എക്സ്-100, കിരിയാത്ത് സത്ത് + പൊങ്കാമിയ എണ്ണ + ട്രൈറ്റൺ എക്സ്-100, കിരിയാത്ത് സത്ത് + ട്രൈറ്റൺ എക്സ്-100 എന്നിവയുടെ 1, 2, 3, 5, 10, 20 ശതമാനം വീര്യമുള്ള ഫോർമുലേഷനുകൾ ഇലകളിൽ തളിച്ചതിനു ശേഷം സി.ഐ.ബി.ആർ.സി (സെൻട്രൽ കീടനാശിനി ബോർഡും രജിസ്ട്രേഷൻ കമ്മിറ്റി) നടപടി ക്രമം അനുസരിച്ച് ഇലകളിൽ മഞ്ഞനിറം, കരിച്ചിൽ, നെക്രോസിസ്, എപിനാസ്റ്റി, ഹൈപ്പോനാസ്റ്റി എന്നിവ ഉൾപ്പെടെയുള്ള ലക്ഷണങ്ങളുടെ നിരീക്ഷണങ്ങൾ നടത്തുകയും തരംതിരിക്കുകയും ചെയ്തു. 1 മുതൽ 20 ശതമാനം പരിധിക്കുള്ളിൽ വീര്യമുള്ള എണ്ണ

അധിഷ്ഠിത കിരിയാത്ത് ഫോർമുലേഷനുകൾ പയറിൽ ഒരു തരത്തിലുള്ള ഫൈറ്റോടോക്സിക് പ്രതികരണവും ഉണ്ടാക്കുന്നില്ലെന്ന് ഫലങ്ങൾ വെളിപ്പെടുത്തി.

പ്രസ്തുത ഗവേഷണ ഫലത്തെ താഴെ വിവരിക്കുന്ന രീതിയിൽ ചുരുക്കി പ്രസ്താവിക്കുന്നതാണ്. പവയറിന്റെ നീരൂറ്റി കുടിക്കുന്ന കീടങ്ങളുടെ പരിസ്ഥിതി സൗഹാർദ്ദപരമായ നിയന്ത്രണത്തിനായി, 6 ശതമാനം വീര്യമുള്ള കിരിയാത്ത് സത്ത് + വേപ്പ് എണ്ണ + ട്രൈറ്റൺഎക്സ്-100 (7:2:1) സംയോജിപ്പിച്ചു ഉണ്ടാക്കിയ ജൈവകീടനാശിനി രണ്ടാഴ്ച ഇടവേളകളിൽ പ്രയോഗിക്കുമ്പോൾ അവയുടെ ഫലപ്രാപ്തി രാസ ചികിത്സയ്ക്കു തുല്യമാണെന്ന് കണ്ടെത്തി.

APPENDIX-I

Weather data during the cropping period

Month	Temperature(°C)		Relative humidity (%)		Rainfall (mm)
	Max	Min	Max	Min	
June-19	31.91	25.37	90.40	77.96	10.66
July-19	30.89	24.90	91.41	79.8	5.89
August-19	30.75	24.24	92.03	77.9	10.70
September-19	30.99	24.43	91.3	78.03	9.32
October-19	30.76	24.13	92.83	77.09	13.06
November-19	31.9	24.54	91.50	77.93	3.04
December-19	31.91	23.83	92.22	77.13	6.48
January-20	32.31	22.92	92.48	63.62	1.77
February-20	33.04	23.25	90.31	60.75	0.00
March-20	33.60	24.75	87.55	59.98	2.10
April-20	34.24	25.76	84.85	62.29	2.65