POTENTIAL OF THE NATURAL BIO POLYMERS, CHITIN AND CHITOSAN IN PEST MANAGEMENT

by

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(2015-11-114)

THESIS

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2017

DECLARATION

I, hereby declare that this thesis entitled **"Potential of the natural bio polymers, chitin and chitosan in pest management"** 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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LIST OF ABBREVIATIONS AND SYMBOLS USED

@	At the rate of
®	Registered
%	Per cent
µg	Micro gram
ANOVA	Analysis of Variance
a.i	Active ingredient
CD	Critical difference
CRD	Completely Randomised Design
cm	Centimetre
cm^3	Cubic centimetre
DAI	Days after inoculation
DAT	Days after treatment
et al.	and co-workers/co-authors
EC	Emulsifiable concentrate
Fig.	Figure
G	Granule
G	Gram
$g\ kg^{-1}$	Gram per kilogram
HAT	Hour after treatment
H	Hours
ha	Hectare
ha^{-1}	Per hectare
$harvest^{-1}$	Per harvest
i.e.	That is
Kg	Kilogram
$Kg\ ha^{-1}$	Kilogram per hectare
KAU	Kerala Agricultural University
K Da	Kilo Dalton
L	Litre
LC_{50}	Lethal dose required for killing 50 per cent of test insect

Ltd	Limited
L^{-1}	Per litre
M	Molarity
M	Meter
mL	Millilitre
mL^{-1}	Per millilitre
Min	Minutes
mol^{-1}	Per molecular weight
N	Normality
N	Total number
No.	Number
NA	Not analysed
NS	Non significant
pH	Negative logarithm of hydrogen ion concentration
Plant $^{-1}$	Per plant
ppm	Parts per million
RBD	Randomised Block Design
Sl. no	Serial number
sp.	Species
WAT	Weeks after treatment
viz.,	Namely

Introduction

1. INTRODUCTION

Present day agriculture prefers bio-based materials for a safe future. Therefore there is an increasing awareness on the suitability of using materials like natural biopolymers for diversified applications in life science (Tharanathan, 2003). The exploitation of such bioactives that are compatible with the environment is one of the main challenges in modern agriculture.

Biopolymers are a class of extremely active polysaccharides. Natural biopolymers have diversified applications, because of the advantages like availability, biocompatibility, biodegradability and ecological safety. There are three main classes of biopolymers, based on the monomeric units used and the structure of the biopolymer formed. They are polynucleotides (RNA and DNA), which are long polymers composed of 13 or more nucleotide monomers; polypeptides which are short polymers of amino acids and polysaccharides which are often linear bonded polymeric carbohydrate structures.

Cellulose and chitin are the main natural polysaccharides on earth, of which chitin is the second most abundant natural polysaccharide on the planet, next to cellulose. With an annual estimate of at least 1×10^9 tons, chitin is being synthesized and degraded every year in nature because of high regeneration rate (Gooday, 1990). Chitosan, a deacetylated derivative of chitin is also biodegradable, biocompatible and is non toxic to mammals with LD_{50} to mice $> 16\ kg^{-1}$ (Singla and Chawla, 2001). The degree of N - acetylation and molecular weight are important factors that have an impact on its biological activity (Rabea *et al.*, 2003; Gerasimenko *et al.*, 2004; Badawy, 2010).

Utilization of these biopolymers in agriculture as biostimulants, will help to reduce the quantity of fertilizers and plant protection chemicals as well as to elicit more healthier and sustainable organic agriculture (Cabrera *et al.*, 2013). They are biologically active during their interaction with plants and microorganisms and hence can be exploited for protection of plants from pests

and diseases before and after harvest. These biopolymers enhance the action of biocontrol agents as well and augment the symbiotic interaction between plants and beneficial microorganisms (Hirano, 1997).

Due to their insecticidal and fungicidal properties they serve as good alternatives for broad - spectrum and highly persistent pesticides. Moreover chitinous amendments were reported to have impressive reductions in the levels of the phytopathogenic nematodes as well (Mian *et al.*, 1982; Godoy *et al.*, 1983) suggesting that chitin acts as a prebiotic, which can promote the growth of beneficial chitinolytic microbes (Rodriguez - Kabana *et al.*, 1987). This lead to the development of chitin derivatives for nematode management. Apart from their insecticidal, fungicidal and nematicidal properties, they have plant growth promoting attributes too. Furthermore, they also act as antiviral, anti fungal and anti bacterial by inducing plant defense (Xing *et al.*, 2015).

Chitin and chitosan are non-toxic to vertebrates and humans. The Environmental Protection Agency (EPA) concluded that they do not pose any major identifiable risks to human health (EPA, 2008).

The insecticidal properties of chitin and chitosan make them a novel tool in pest management strategy. Perusal of literature revealed that products based on these may serve as good alternatives for broad-spectrum and highly persistent pesticides. Their insecticidal properties on homopteran insects was studied by Casals *et al.* (2002) and their activity on some lepidopteran insects was demonstrated by Zhang *et al.* (2003).

The afore mentioned facts were much persuading to take up an investigation to evaluate their efficacy in managing some of the economically important pests of crop plants including insects and nematodes. Therefore, the present investigation was phased out with the following objectives:

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- Laboratory screening of insect and nematode pests for their vulnerability to chitin and chitosan formulations
- Pot culture experiment to study their impact on pest population build up, disease incidence and growth parameters
- Field experiment to assess the efficacy of the formulations in managing the pests as well as their impact on crop yield.
- Safety evaluation on natural enemies of insect pests

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Review of Literature

2. REVIEW OF LITERATURE

Exploitation of environment friendly bioactives is a major challenge in the present day agricultural scenario. Chitin is the second most abundant polysaccaride and a renewable biopolymer, next to cellulose (Rabea *et al.,* 2003). They are basically the by products of crab and shrimp canning industry. Goycolea *et al.* (2004), estimated that 1, 70, 000 ton of chitinous wastes are obtained per year from fish industry annually. One of the most relevant derivatives of chitin is chitosan, which is soluble in dilute acids (Prashanth and Tharanathan, 2007: Xu *et al.*, 2008).

2.1 Potential of Chitin and Chitosan in Plant Protection

2.1.1 In Pest Management

2.1.1.1 Insect pests

2.1.1.1.1 Chitin

The potential of direct use of chitin in pest management is less studied, as per perusal of literature. Chitin and its derivatives are promising alternatives to inorganic pesticides because of their biological activity, biodegradability, biocompatibility, nontoxicity, adsorption and availability (Zong *et al.,* 2000). Nevertheless, its utilization for improving the performance of biocontrol agents, especially the entomopathogens, are seen evaluated by various researchers. Senthilraja *et al.* (2010) reported that, foliar application of talc based formulations of *Beauveria bassiana* (Balsamo) Vuillemin and *Pseudomonas fluorescens* (Flugge) Migula, amended with chitin 1% significantly reduced the incidence of groundnut leaf miner, *Aproaerema modicella* Dev. from 31.5 to 2.5 per cent. Nithya (2015) found that spores of *Lecanicillium lecanii* (Zimm.) Zare and Gams harvested from Sabouraud Dextrose Broath (SDB) enriched with 5% chitin, caused 100 per cent mortality of cowpea aphid *Aphis craccivora* Koch at 72 hours after treatment (HAT), when compared to non enriched SDB where 59 per cent mortality was recorded. Similar result was also reported by Jasmy (2016) who

found that SDB amended with 0.3% chitin, recorded 93.66 per cent mortality of brinjal mealy bug *Coccidohysterix insolitus* Green compared to 85.33 per cent mortality obtained with non enriched medium at 24 HAT.

Sankar (2017) reported that, chitin enriched bioformulations of *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno and *L. lecanii* @ 10^7 spores mL^{-1} caused 20 to 100 per cent mortality in rice hoppers *viz.*, brown plant hopper *Nilaparvata lugens* Stal, white leaf hopper *Cofana spectra* Distant and white - winged plant hopper *Nisia nervosa* Motschulsky, *in vitro* at 48 HAT. He also found that chitin enriched *L. saksenae* recorded complete mortality of rice bug *Leptocorisa acuta* (Thunberg) at 72 HAT.

2.1.1.1.2 Chitosan

Chitosan, the deacetylated form of chitin was reported to have insecticidal properties, by various workers.

The insecticidal activity of chitosan on gram pod borer, *Helicoverpa armigera* Hubner and Diamond back moth *Plutella xylostella* L. was tested by Zhang *et al.* (2003). They observed that cole leaves sprayed with 0.3 per cent chitosan solution resulted in 40 and 72 per cent mortality respectively, at 72 HAT. They also found that application of chitosan @ 6 to 60 g L^{-1} on flowers resulted in 93 to 99 per cent mortality of mealy plum aphid *Hyalopterus pruni* Goffroy and 70 to 80 per cent of mortality in corn leaf aphid *Rhopalosiphum padi*, L., rose - grain aphid *Metopolophium dirhodum* Walker and cotton aphid *Aphis gossypii* Glover.

Rabea *et al.* (2005) reported that chemically modified form of chitosan was more effective on *Spodoptera littoralis* Boisduval larvae compared to its original forms. Artificial diets of third instar larvae of *S. littoralis* mixed with O - (butyroyl) chitosan, O - (2 - methylbutyroyl) chitosan, O - (bentanoyl)

chitosan and O - (heptanoyl) chitosan @ 5 g kg^{-1} resulted 10 to 57 per cent of mortality, five days after treatment (DAT), whereas the corresponding mortality of larvae treated with original form was only 10 per cent. Similar results were also reported by Rabea *et al.* (2006). They found that, artificial diet mixed with *N* - (3 - phenylbutyl) chitosan, *N* - (tridecanyl) chitosan and *N* - (2 - phenylethyl) chitosan @ 5 g kg^{-1} resulted in 50, 47 and 37 per cent mortality respectively in third instar larvae of *S. littoralis* at five DAT.

Saadiya *et al.* (2011) carried out histological studies on third instar larvae of *Galleria melleonella* L. fed with artificial diets amended with chitosan. Their studies revealed the presence of elongated, disorganised and disintegrated mid gut epithelia in the treated larvae. Performance of chitosan having varying molecular weights was compared by Badawy and El-Aswad (2012). Chitosan, with molecular weights, 2.27×10^5, 3.60×10^5, 5.97×10^5, and 9.47×10^5 g mol^{-1} were evaluated along with their various metal complexes like Ag (I), Cu (II), Ni (II), and Hg (II). They found that artificial diet incorporated with chitosan, 2.27×10^5 g mol^{-1} and its complexes with Ni and Hg @ 4 g a.i. kg^{-1} resulted in maximum growth inhibition (77.8, 97.3 and 96.2 per cent respectively), feeding inhibition (76, 90.2 and 86.8 per cent respectively) and mortality (50, 93.3 and 83.3 per cent, respectively) in third instar larvae of *S. littoralis*. Similar results were also reported in oleander aphid *Aphis nerii* Boyer de Fonscolombe by leaf dip and bioassay methods. After 24 hours, 3.60×10^5, 5.97×10^5 and $9.47\text{x}10^5$g mol^{-1} of chitosan caused 48, 49, and 46 per cent mortality in leaf dip method while in bioassay the mortality was 96, 87 and 100 per cent respectively. Among the metal complexes, chitosan - Cu complex recorded 70, 73 and 94 per cent of mortality of aphids at 250, 500 and 1000 mg L^{-1}.

Zeng *et al.* (2012) conducted detailed investigation on the antifeedant effect of chitosan on black cut worm, *Agrotis ipsilon* Hufnagel and pod borer *Maruca vitrata* Fabricius of soyabean. They found that artificial diets mixed with chitosan at 5 g kg^{-1} recorded highest Antifeedent Rates (AR) of 82.89 and 87.24

per cent, respectively. They also observed that soyabean seeds coated with chitosan in the ratio of 1: 50 (w/w) significantly increased the germination to 90 per cent and yield by 20 per cent. The increase in yield was attributed to the reduction in pest incidence.

Bharani *et al.* (2014) evaluated the insecticidal activity of chitosan nanoparticles incorporated with Beauvericin (Csnp - Bv) formulation on *S. litura* and observed that, there was 100 per cent mortality of larvae when treated with 1.0, 0.01 and 0.001 mg concentrations, in the first and second instars and that the per cent mortality decreased with increase in size, reaching 24, 11.2 and 3.0 per cent in the sixth instar, with each of the concentrations respectively.

The effect of chitosan on coleopteran pests was first reported by Sahab *et al.* (2015). They observed that artificial diet mixed with 12.5 parts of chitosan (CS) - g - poly acrylic acid (PAA) nano particles significantly reduced the fecundity of cowpea weevil, *Callosobruchus maculates* F. from 95.3 to 10.9 *in vitro* and from 94.3 to 19.9 under storage. It was also found to suppress the growth of weevils by 65 per cent in laboratory and 71 per cent in storage. Similar reduction in fecundity was observed in *Callosobruchus chinensis* L. where the reduction was from 96.3 to 21.9 and 91.3 to 21.1 per cent respectively under laboratory and storage. Another work carried out by them revealed that diet containing 12.5 parts of chitosan decreased the fecundity of *A. gossypii* from 97.3 to 20.9 and 90. 3 to 28.9, under laboratory and semi field conditions. They also reported a reduction in larval weight by 77.8 per cent.

2.1.1.2 Nematode pests

2.1.1.2.1 Chitin

Considering the environmental and economic reasons, management of plant parasitic nematodes using chemicals is not a viable option. Recent strategies include the use of soil amendments, resistant or tolerant plants or a combination of

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these. Chitin, the most commonly occurring nitrogen - containing polysaccharide in nature, has been used as a soil amendment to control root - parasitic nematodes (Alexander, 1977).

Nematicidal activity of chitin on *Meloidogyne incognita* Kofoid and White) Chitwood was first reported by Mankau and Das (1969). Mian *et al.* (1982) found that soil amended with 2 - 4% chitin did not record any *Meloidogyne arenaria* Chitwood galls in summer crookneck squash, *Cucurbita pepo* L. Rodriguez - Kabana *et al.* (1984) reported that, soil mixed with chitin (0.5 - 4 w/w) reduced the plant parasitic nematodes *viz.*, *Heterodera glycines* Ichinohe, *Helicotylenchus dihystera* (Cobb) Sher and *M. incognita.* They also found that it also increased the population of non phytoparasitic nematodes, soil microflora and enzymatic activity, eight weeks after treatment. Niether the population nor the galls of *M. arenaria* was reported in soil amended with mixture of chitin 2% and hemicelluloses (0.5 - 2%) in protected condition (Culbreath *et al.*, 1985).

Spiegel *et al.* (1986) observed a reduction in gall index of *M. javanica* in bean and tomato plants under pot culture when the potting mixture was amended with ClandoSan 0.05 to 0.3 %, a commercial formulation of chitin. Spiegel *et al.* (1989) also reported 50 per cent reduction in the population of cereal cyst nematode *Heterodera avanae* Wollenweber in wheat and 90 per cent reduction in *Tylenchulus semipenetrans* Cobb in citrus.

Westerdahl *et al.* (1992) reported a reduction in population of juveniles and the gall rating of *M .incognita* in the root zone of tomato, when the soil was amended with ClandoSan @ 1,093 kg ha^{-1}. The juvenile population was reduced from 100 to 13 and the gall rating from 0.45 to 0. They observed that in soil amended with ClandoSan @ 1,893 kg ha^{-1}, reduced the population of lesion nematode, *Pratylenchus neglectus* Rensch from 1779 to 1065, two months after application, in walnut. Belair and Tremblay (1995) reported that, *M. hapla*

infested soil, when mixed with ClandoSan 0.4% increased the root weight by 186 per cent and leaf weight by 37.59 per cent in tomato, under protected cultivation. The tissues of chitin - urea amended plants were observed to contain 10 per cent increase in concentration of Ca, N, B, Fe and Zn.

Potting mixture added with 0.5 g of chitin and *Paecilomyces lilacinus* (Thom) Samson spores decreased the number of galls from 330.67 to 9.84 in brinjal, 80.82 to 3.51 in tomato and 70.92 to 1.42 in bengal gram, 90 days after inoculation (Mittal *et al.*, 1995). Soil amended with chitin 1% (w/w) decreased the population of plant parasitic nematodes and increased the saprophytic nematodes in cowpea (Khan and Saxena, 1997). Hallmann *et al.* (1999) reported 95 per cent increase in shoot weight and 100 per cent decrease in gall index of *M. incognita,* in cotton. They also reported an increase in microbial population as well as chitinolytic activity in soil.

Soil amendment with chitin 1% notably reduced the population of *Heterodera trifolii,* Goffart by 38.46 per cent and *Pratylenchus* sp. by 50 per cent in white clover roots. There was 55.55 per cent reduction of *H. trifolii* and 99.73 per cent reduction of *Paratrichodorus minor* (Colbran) Siddiqi in soil of ryegrass, (Bell *et al.,* 2000). De Jin *et al.* (2005) found that soil amended with chitin compost and chitin broth reduced the gall index of *M. incognita* after 4, 6, and 8 weeks of inoculation, in tomato.

Ladner *et al.* (2008) reported significant decrease in number of eggs and juveniles (J_2) when 100 g and 200 g of the commercial formulation of chitin, Ecologic® was incorporated in soil against root knot nematode of tomato. In an experiment with rape seed, Korayem *et al.,* (2008) observed that chitin @ 8 g m^{-2} was found to reduce 75.4 per cent of *M. incognita* galls, 84.8 per cent of females and 94.7 per cent juvenile population. They also reported enhancement in root length by 10.3 per cent and shoot weight by 26 per cent.

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Kalaiarasan *et al.* (2008) reported that potting mixture amended with chitin 1% reduced *M. arenaria* population by 25.4 per cent, galls per plant by 41.3 per cent and egg masses per plant by 9.5 per cent, in groundnut. Potting mixture amended with crab shell waste of 1.27 and 1.63 g kg^{-1} considerably reduced the juvenile population by 70.3 and 72.5 per cent, as well as gall index by 58.7 and 59.5 per cent in tomato (Saad *et al.*, 2012).

2.1.1.2.2 Chitosan

Radwan *et al.* (2012) reported that potting mixture amended with chitin and chitosan at different doses *viz.*, 1, 3, 5, 10 g kg^{-1} of the soil, prior to planting reduced the *M. incognita* galls by 58.29 per cent and J_2 by 51.43 per cent. Osman *et al.* (2013) evaluated the nematicidal and enzymatic activity of chitosan with foliar spray and root dip treatment and observed that chitosan @ 2500 ppm increased the activity of the enzymes, peroxidase by 375 per cent, polyphenol oxidase by 600 per cent and chitinase by 281 per cent. Escuderoa *et al.* (2016) found that chitosan increased egg parasitisation by nematophagous and root endophytic fungus, *Pochonia chlamydosporia* (Goddard) Zare and Gams, in *M. javanica.*

2.1.2 In Disease Management

2.1.2.1 Chitin

Bell *et al.* (1998) reported that addition of chitosan 3 mg mL^{-1} inhibited the mycelial growth of the plant pathogenic fungi *Fusarium oxysporum* f. sp. *apii* (Nelson and Scherb) both *in vitro* and *in vivo,* in celery. Soils treated with chitin plus chitosan, drastically reduced the incidence by 61.6 per cent at 60 DAT and 23.6 per cent at 90 DAT. Abdel-Fattah and Mohamedin (2000) studied the effect of interaction between a vesicular - arbuscular - mycorrhiza (VAM) and *Streptomyces coelicolor* (Muller) Waksman and Henrici, in soil amended with

chitin waste in sorghum and reported that the intensity of infection was reduced by 30 per cent.

El - Moughy *et al.* (2006) observed that potting mixture consisting of 6 g kg^{-1} of chitin and chitosan drastically decreased the root rot of tomato by *Rhizoctonia solani* Kuhn, *Fusarium solani* (Mart.) Sacc. and *Sclerotium rolfsii* Sacc. irrespective of the method of treatment *viz.*, soil amendment, seed bed treatment and seedling dip methods. The per cent reduction in disease incidence ranged from 70.8 to 89.1 per cent in different methods of treatment. They also observed that incorporation of a mixture of chitin and chitosan @ 6 g kg^{-1} of soil reduced the disease incidence by 91 per cent, and increased the yield by 66.7 per cent.

2.1.2.2 Chitosan

Anti microbial activity of chitosan was reported by Ghaouth *et al.* (1992), where complete inhibition of mycelial growth of *Pythium aphanidermatum* (Edson) Fitzp was observed in media amended with 400 µg mL^{-1} of chitosan. Sathiyabama and Balasubramanian, (1998) tested different concentrations of chitosan on germination of *Puccinia arachidis* Speg. and found that none of the uredospores germinated in 1000 ppm chitosan.

In an *in vitro* study conducted by Ben - Shalom *et al.* (2003) proved that chitosan at 50 ppm completely inhibited the germination of *Botrytis cinerea* Pers. conidia. They also observed that potted cucumber plants sprayed with 0.1% chitosan at 24 hour before inoculation reduced the disease index (0.45) when compared to control (3.5).

Kowalski *et al.* (2007) found that, combined foliar application and seed tuber treatment in potato with chitosan @ 5 g L^{-1} increased the yield by 14.58 per cent and reduced the incidence of late blight by 3.15 per cent.

Growth inhibition of *Alternaria solani* (Ellis and Martin) Sorauer, to the tune of 62.3 to 68.6 per cent was observed in tomato plants when treated with chitosan @ 1 to 5 mg mL^{-1} (Sathiyabama *et al.*, 2014). Tobacco plants treated with 50 µg mL^{-1} of oligochitosan reduced the intensity of *tobacco mosaic virus* and reduced the incidence by 85.45 per cent.

Moret *et al.* (2009) reported that cucumber plants treated with chitosan 2.5% significantly reduced the incidence of *Sphaerotheca fuliginea* Schlecht by 17.04 to 65 per cent and *Erysiphe cichoracearum* DC. by 53.75 per cent when the cotyledons were inoculated with $4x10^5$ spore ml^{-1}.

Cucumber seeds when treated with 85 per cent deacetylated chitosan increased the germination by 76.9 per cent due to inhibition of the damping - off pathogen, *Pythium aphanidermatum* (Li *et al.*, 2011). Foliar application of chitosan combined with salicylic acid, after 14 and 30 days after inoculation reduced the mosaic symptoms and the symptoms exhibited later compared to uninoculated plants. It was also found to significantly increase N, P, K, Fe and Zn concentrations in tomato (El - Gawad and Bondok, 2015).

Nanoparticles of chitosan - silver @ 10 mg, 20 mg and 30 mg significantly inhibited the growth of *Streptococcus* sp. (Devadiga *et al.*, 2016).

2.1.3 Effect on Entomopathogens

2.1.3.1 Chitin

Nandakumar *et al.* (2007) observed that addition of chitin 1% in the liquid medium inoculated with *Pseudomonas fluorescens*, increased the beneficial bacterial antagonists.

Nithya (2015) proved that chitin (2 to 5%) amended media could significantly increase sporulation of *L. lecanii* by ten fold. Jasmy (2016) reported an increase in number of colony forming units of *L. saksanae* when the culture media *viz*., rice bran and SDB was amended with chitin 5%.

2.1.3.2 Chitosan

Palma-Guerrero *et al.* (2008) found that chitosan @ 0.01mg mL^{-1} increased the sporulation and viability of entomopathogenic fungus *B. bassiana*, that renders chitosan as a suitable compound to increase the conidiation. Jasmy (2016) reported a drastic increase in spore yield and number of colonies of *L. saksenae* when the culture media rice bran and wheat bran was enriched with chitosan 5%.

2.1.4 Growth Promoting Characters of Chitin and Chitosan

2.1.4.1 Chitin

Khan *et al.* (2002) reported that foliar application of chitin pentamer 10^{-5} to 10^{-7} increased the photosynthetic rate by 8 to 10 per cent over control in maize on second day after treatment. Muymas *et al.* (2015) reported that soil amended with 20% fermented chitinous material increased the total N by 0.34 per cent and P availability in soil by 549 mg kg^{-1}.

2.1.4.2 Chitosan

Khan *et al.* (2002) evaluated different concentrations (10^{-5} and 10^{-7}) of chitosan pentamer in maize and soyabean, and found that three DAT, there was an increase in the net photosynthetic rate by 18 and 10 per cent respectively, over control.

Burrows *et al.* (2007) found that, 0.5% HCl demineralised chitosan treated seeds exposed for 30 min before planting, recorded highest germination of

90 per cent in pea nut, whereas chitosan demineralised with 1% HCl and 5% CH_3COOH significantly increased the average number of leaves by 82.7 and 68.8 per cent as well as plant height by 58.45 and 48.92 per cent.

Foliar application of chitosan 0.05% in cucumber resulted in 41.52 per cent increase in plant height, 66.5 per cent in branches, 63.82 per cent in shoot weight and 46.33 per cent leaf area as well as 70.18 per cent increase in yield over control (Farouk *et al.*, 2008).

In sweet pepper, spraying of 6 g L^{-1} of commercial formulation of chitosan, Chitocare® increased the plant height by 77.69 per cent, number of leaves by 172 per cent, branches by 120 per cent and yield by 140 per cent (Ghoname *et al.*, 2010). In radish, high cadmium level soil amended with 200 mg kg^{-1} of chitosan notably increased the shoot fresh weight by 54.25 per cent, dry weight of shoot by 92.76 per cent, number of leaves by 46.99 per cent and shoot length by 23.3 per cent and root length by 23.15 per cent, over untreated check (Farouk *et al.*, 2011).

Sheikha and AL-Malki (2011), reported that application of chitosan 0.5% through irrigation enhanced the root length by 32.78 per cent in cowpea. They found that, application of chitosan 2.5 % increased the fresh shoot weight by 6.76 per cent and the root weight by 8.13 per cent. In a pot culture experiment in cowpea, Farouk and Amany (2012) observed that foliar application of chitosan @ 250 mg L^{-1}, increased 15.2 per cent plant growth, 27.11 per cent leaves, 46.25 per cent branches and 26.66 per cent yield.

Mondal *et al.* (2012) found that, spraying of chitosan at 125 ppm on okra at 25, 40 and 55 days after sowing increased the plant height by 17.53 per cent. It was found to increase the number of leaves by 42.85 per cent and yield by 27.90 per cent. El - Miniawy *et al.* (2013) reported that application of chitosan @ 5ml

L^{-1} increased 27.67 per cent of plant height, 25.38 per cent of leaves, 17.68 per cent of leaf area and yield by 21.93 per cent, in strawberry.

Salachna and Zawadzinska (2014) evaluated the different molecular weight chitosan and found that application of high molecular weight chitosan (970 k Da) increased the plant height by 19.33 per cent, leaves by 55.64 per cent and shoots by 25 per cent in corm dip method. Application of chitosan @ 75 mg L^{-1} in tomato was found to significantly increase the plant height by 35.61 per cent, number of branches by 38.05 per cent, leaf area by 24.79 per cent and yield by 43.64 per cent (Mondal *et al.*, 2016).

2.1.5 Safety to Non Target organisms

New interventions in ecosystem using bio based materials needs risk evaluation to avoid adverse impacts on environment. Since utilization of the biopolymers, chitin and chitosan in agriculture is in its infancy, not much works could be cited regarding its safety aspects. However, the Environmental Protection Agency (EPA) (2008) concluded chitin and chitosan were non toxic to human health. Palma - Guerrero *et al.* (2010) proved that chitin and chitosan were more toxic to phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Jarvis and Shoemaker) than entomopathogenic fungi *B. bassiana* and nematophagous fungus, *P. clamydosporia* on the basis of permeability of plasma membrane. They found that, the difference in membrane fluidity of chitosan might be due to the presence of quantity of polyunsaturated fatty acids in plasma membranes.

Sankar (2017) found that chitin enriched bioformulations of *L. saksenae* and *L. lecanii* (10^9 spores mL^{-1}) were safe to natural enemies *viz.*, the coccinellids, *Micraspis discolor* F. and *Coccinella transversalis* F., the adults of the mirid, *Cyrtorhinus lividipennis* Reuter, the carabid, *Ophionea nigrofasciata* Schmidt-Gobel, and the spiders *Tetragnatha maxillosa* Thorell and *Oxyopes shweta* Tikader.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Potential of the natural biopolymers, chitin and chitosan in pest management" was carried out at the Biocontrol laboratory for crop pest management, Department of Agricultural Entomology, College of Agriculture, Vellayani. Pot culture studies and field experiments were conducted at the Instructional farm, College of Agriculture, Vellayani.

3.1 LABORATORY SCREENING AGAINST MAJOR INSECT PESTS, PLANT PARASITIC NEMATODES AND NATURAL ENEMIES

To study the effect of the natural biopolymers, chitin and chitosan on insect pests, plant parasitic nematodes and natural enemies, the following treatments and test organisms were selected.

Treatments

T1- Chitin 3% - 5 g of colloidal chitin in 100 mL of water

T2 - Chitin 5% - 5 g of colloidal chitin in 100 mL of water

T3 - Chitin 7% - 7 g of colloidal chitin in 100 mL of water

T4 - Chitosan 3% - 3 g of colloidal chitosan in 100 mL of water

T5 - Chitosan 5% - 5 g of colloidal chitosan in 100 mL of water

T6 - Chitosan 7% - 7 g of colloidal chitosan in 100 mL of water

T7 - Chitosan gel 3% - 0.99 g of 30% chitosan gel in 100 mL of warm water

T8 - Chitosan gel 5% - 1.65 g of 30% chitosan gel in 100 mL of warm water

T9 - Chitosan gel 7% - 2.31 g of 30% chitosan gel in 100 mL of warm water

T10 - Bioboost 2% (chitosan based) - 2 mL per 100 mL of water

T11 - Biorakshak 2% (chitin based) - 2 mL per 100 mL of water

T12 - Control - Sterile water

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Test organisms selected were insect pests belonging to three different orders, plant parasitic nematodes under three different genera and the three major group of insect predators *viz.,* the coccinellids, syrphids and spiders. The lepidoteran insects tested were the pumpkin caterpillar, *Diaphania indica* Saunders and the cut worm, *Spodoptera litura* F., the coleopteran pests were the leaf beetle, *Henosepilachna vigintioctopunctata* F. and the leaf weevil, *Myllocerus viridanus* Schoenherr and the hemipteran pests tested were *Aphis craccivora* Koch and *Riptortus pedestris* F. The plant parasitic nematodes screened were the root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood., the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira and the spiral nematode, *Helicotylenchus* sp. The predatory coccinellids, *Chilomenes sexmaculata* F., *Coccinella transversalis* F., the Syrphids, *Ischiodon scutellare* F., *Xanthogramma scutellare* Thorell and the predatory spiders, *Tetragnatha mandibulata* Walckenaer and *Tetragnatha maxillosa* Thorell were the natural enemies screened for susceptibility.

3.1.1 Rearing of Test Organisms

3.1.1.1 Insect Pests

3.1.1.1.1 Lepidoptera

3.1.1.1.1.1 Diaphania indica Saunders

Laboratory culture was maintained following the procedure adopted by Jasmy (2016) with slight modifications. Larvae of *D. indica* collected from bitter gourd field were released into rearing troughs of size 15 x 20 cm provided with fresh bitter gourd leaves which were kept afresh with the help of moistened cotton attached to the detached end of petiole. Fresh leaves were provided on alternative days. The mouth of the jar was covered with a moist muslin cloth tied around. The rearing jars were cleaned every day. Upon pupation, the cocoons were transferred into another glass jar for adult emergence. The newly hatched adults were fed with 10 per cent honey solution added with a drop of vitamin E and were

kept for oviposition. On hatching, the first instar larvae were transferred to new jars using a fine camel hair brush and fed as before, to continue rearing.

3.1.1.1.1.2 Spodoptera litura F.

Egg masses collected from banana plants were surface sterilized and kept for emergence in rearing troughs (15 x 20 cm) covered with muslin cloth. The newly emerged larvae were transferred to another sterile jar along with the leaf and were fed with fresh castor leaves daily. Care was taken to maintain hygiene of the rearing materials so as to avoid viral infection. The number of caterpillars in each jar was maintained in such a way as to avoid overcrowding. Sufficient feed was provided daily to minimize cannibalism. After final moulting larvae were transferred to rearing troughs provided with sterilize soil, for pupation. Emerging adults were released into fresh plastic containers provided with feed mentioned in para 3.1.1.1.1.1. To enable oviposition, the method followed by Anusree (2016) was adopted. Rough folded drawing sheets were provided for oviposition. After oviposition the sheets were separated and kept in a plastic trough covered with muslin cloth, for adult emergence.

3.1.1.1.2 Coleoptera

3.1.1.1.2.1 Henosepilachna vigintioctopunctata F.

Egg masses were collected from the brinjal fields along with the leaves and kept in the 9 cm Petri plates for emergence. The culture was maintained as per the method described by Sharma and Saxena (2007). Newly emerged grubs were released into plastic troughs (15 x 20 cm) provided with tender twigs kept afresh by dipping the cut end in a cotton ball and keeping it immersed in a small glass vial with fresh water. Mouth of the troughs was covered with muslin cloth tied with a rubber band. Shoot tips were replaced on alternate days. The emerging beetles were transferred to another rearing trough to continue their life cycle.

3.1.1.1.2.2 Myllocerus viridanus Schoenherr

Adult weevils were collected from brinjal field and reared using fresh brinjal twigs kept afresh by keeping the twig immersed in a small vial containing water soaked cotton. The mouth of the rearing jar was covered with muslin cloth. Fresh twigs were provided as and when needed.

3.1.1.1.3 Hemiptera

3.1.1.1.3.1 Aphis craccivora Koch

Aphid colonies were located in field and the gravid females were collected from them and released into 25 day old potted cowpea plants using a fine camel hair brush. The newly emerging young ones were transferred to new plants to begin a new culture. The colonies were kept free from predators by removing them as and when noticed.

3.1.1.1.3.2 Riptortus pedestris F.

Adult bugs were collected from the infected cowpea field. Cultures were maintained as per the method described by Nithya (2015). Healthy adults were released into a glass jar (17 x 10 cm) containing tender cowpea pods replaced on alternate days. The eggs laid on the pod surface or bottom of the jar were transferred into a separate jar for emergence. The emerging nymphs were fed as mentioned and the culture was maintained for the experiment.

3.1.1.2 Plant Parasitic Nematodes

3.1.1.2.1 Meloidogyne incognita (Kofoid and White) Chitwood

Tomato plants were raised in grow bags filled with 1:2:1 sterile potting mixture (sand : soil : cowdung). Plants were maintained as per the KAU Package of Practices Recommendations (KAU, 2011). *M. incognita* infected roots were collected from the Department of Nematology, College of Agriculture, Vellayani. Infected roots cut into small bits of five gram were used for inoculating the root zone, by placing it around. Plants were uprooted after 30 days and then washed in

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tap water. Using a sterile sharp needle, the egg masses were transferred into Petri plates containing sterile water.

3.1.1.2.2 Rotylenchulus reniformis Linford and Oliveira

Soil samples from *R. reniformis* infected cowpea rhizosphere were collected and 200 g was processed for extraction of nematodes by Cobb's sieving and decanting method followed by modified Baermann's funnel technique. Cowpea plants were raised as mentioned in para 3.1.1.1.3.1. Fresh nematode culture was added to 10 day old plants near root zone. After one month of inoculation, the soil samples taken from inoculated pots were processed using the above mentioned method.

3.1.1.2.3 Helicotylenchus sp.

Soil samples were collected from *Helicotylenchus* infected banana field and processed by the method mentioned in 3.1.1.2.2. Other nematode species were removed from the suspension and equal number of *Helicotylenchus* (irrespective of stage) was taken for screening.

3.1.1.3 Natural Enemies

3.1.1.3.1 Coccinellids

Laboratory cultures were maintained as per the method adopted by Jasmy (2016). Egg masses of *C. sexmaculata* and *C. transversalis* were collected from aphid colonies and placed in Petri plates (9 cm) for emergence. On hatching the grubs were transferred in to a glass jar provided with aphids colonized on tender cowpea twigs. Pupae were allowed to emerge in the same rearing jar and the adults on emergence were fed with 10 per cent honey solution. Uniform aged adults were selected for testing.

3.1.1.3.2 Syrphids

The pupae of *I. scutellare* and *X. scutellare* were collected from aphid colonies were kept for emergence in the laboratory. Cultures were maintained as per the procedure described by Jasmy (2016). Adults of the two species were maintained separately in rearing troughs (15 x 20 cm) provided with small cotton

bolls soaked in dilute honey kept on the walls of trough. Simultaneously, fresh cowpea twigs having established aphid colonies were kept afresh in a small vial with water soaked cotton plug, to enable oviposition. The colonies were examined for eggs, daily with a magnifying glass. The emerging maggots were transferred to Petri dishes lined with filter paper and fresh twigs of cowpea bearing aphid colonies. The newly emerged adults were fed with aphids to continue their life cycle.

3.1.1.3.3 Spiders

Two spiders *viz.*, *T. mandibulata* and *T. maxillosa* were collected from field and kept under observation for two days.

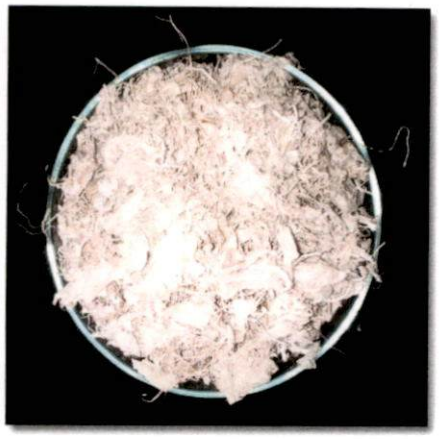
3.1.2 Preparation of Treatment Solutions

3.1.2.1 Colloidal Chitin

Colloidal chitin was prepared based on the method adopted by Hsu and Lockwood (1975), with slight modification. Crude chitin flakes were procured from MATSYAFED, Neendakara, Kollam, Kerala. It was ground in a mixer grinder and 40 g was dissolved in 250 mL of ice cold 0.25 N HCl by intermittently stirring for one hour. After filtration through glass wool the resulting mixture was added drop wise into 2 L of ice cold water with constant stirring using a magnetic stirrer. The white gelatinous precipitate thus obtained was separated by filtering through Whatman No.1 filter paper. The precipitate was washed by re suspending it in one litre of tap water followed by filtration. The process was repeated 5 - 6 times until the p^H of the suspension was neutral (Plate 1).

3.1.2.2 Colloidal Chitosan

Colloidal chitosan was prepared using the method suggested by Fenton and Eveleigh, (1981). Crude chitosan (20 g), procured from MATSYAFED, Neendakara, Kerala, was slowly added into a 0.2 N HCl with continuous stirring and adjusted the p^H into 5.5 by using 0.2 N NaoH and 0.1 N HCl. The resultant



Crude chitin

Deacetylation with HCl

Filtration trough glass wool

Separation

Colloidal chitin after filtration

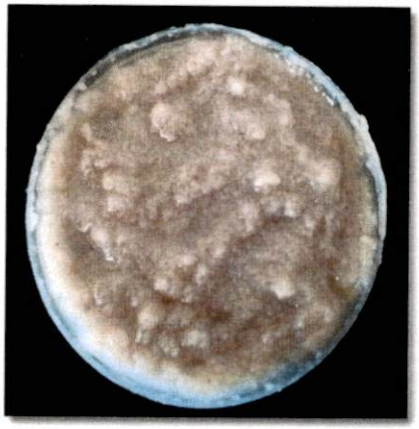
Dried colloidal chitin

Plate 1. Preparation of colloidal chitin

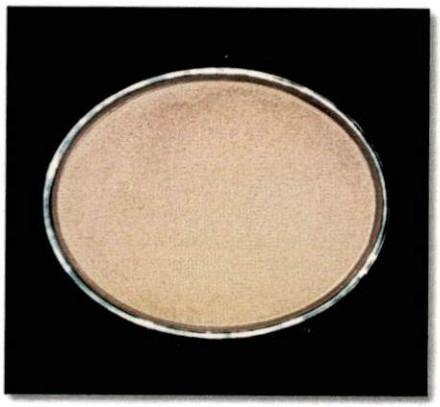
Crude chitosan

Crude chitosan + Hcl

Filtration



Colloidal chitosan



Dried colloidal chitosan

Plate 2. Preparation of colloidal chitosan

white gelatinous material was filtered through a Whatmann No .1 filter paper and the filtrate was dried (Plate 2).

3.1.2.3 Chitosan gel

Chitosan gel (30 %) (Plate 3A) was procured from Pelican Biotech & Chemical Labs (P) Ltd. Alappuzha, Kerala.

3.1.2.4 *Commercial Formulations*

The chitin based formulation, Biorakshak and chitosan based formulation, Bioboost (Plate 3B, C) were purchased from Pelican Biotech & Chemical Labs (P) Ltd. Alappuzha, Kerala.

3.1.3 Screening of Test Organisms

To study the antifeedant and insecticidal properties of chitin and chitosan based bioformulations, experiments were conducted by two methods of treatment. Leaf dip method was followed for studying the antifeedant effect and spray method for insecticidal effect.

3.1.3.1 Insect Pests

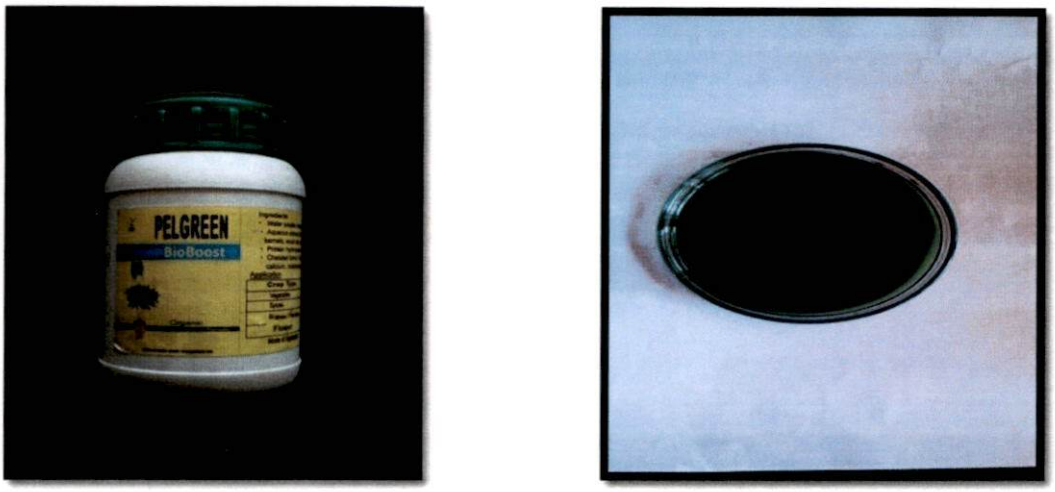
3.1.3.1.1 Lepidoptera

3.1.3.1.1.1 D. indica

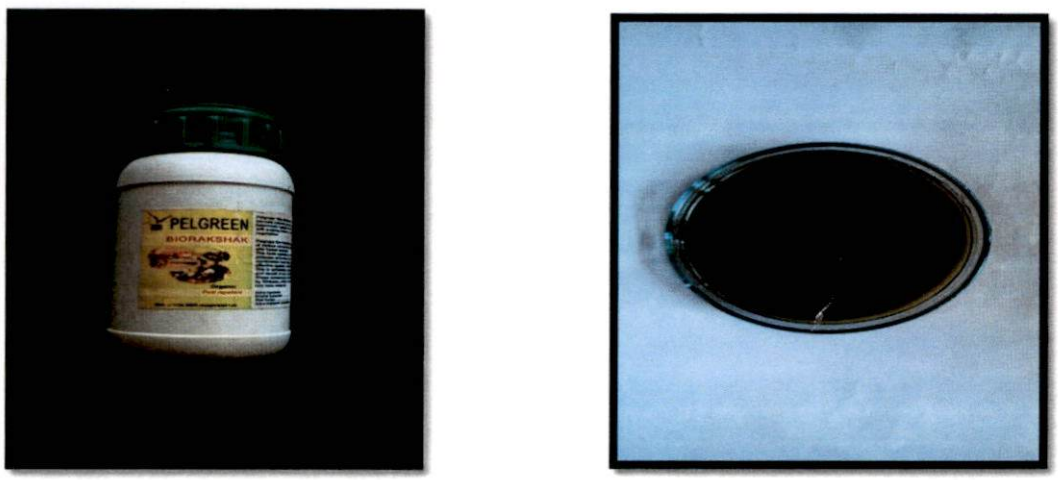
3.1.3.1.1.1.1 Leaf dip method

Uniform sized fresh bitter gourd leaves collected from field which was not sprayed with any chemicals was utilized for the experiment. The leaf area was measured using a graph paper and leaves were dipped separately in treatment solutions for 10 min and air dried for five to 10 min. Leaves were kept afresh by placing on wet cotton pads placed in sterilized Petri plates (Plate 4). Uniform sized second instar larvae (4 day old), pre starved for two hours were released at the rate of 10 per Petri plate and three such plates served as replications. Larvae fed with untreated leaf discs served as control. Observations were recorded at 24 h

A. Chitosan gel 30 %



B. Bioboost (Chitosan based)



C. Biorakshak (chitin based)

Plate 3. Chitin and chitosan based commercial formulations

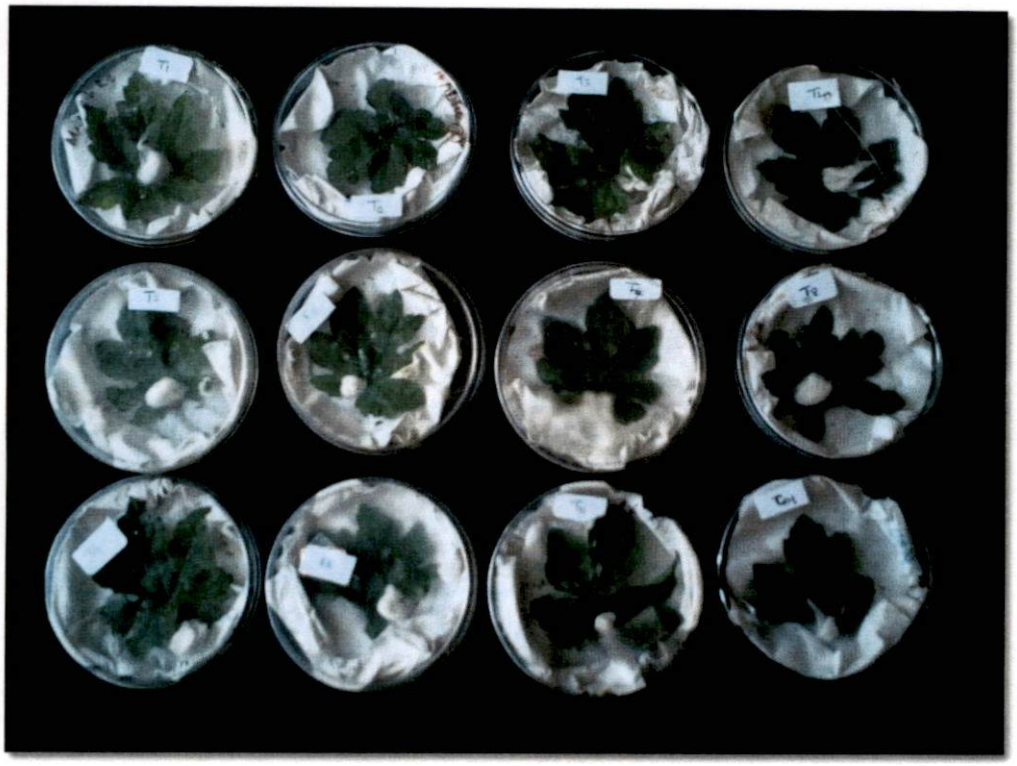


Plate 4. Leaf dip method

interval on behavioural changes and feeding inhibition till they pupated. Feeding inhibition percentage was calculated using the following formula suggested by Arivoli and Tennyson (2013).

$$\text{Per cent antifeedant activity} = \frac{C - T}{C + T} \times 100$$

C - Leaf disc consumed by the larvae in control (cm)

T - Leaf disc consumed by the larvae in treatment (cm)

3.1.3.1.1.1.2 Spray method

Spray method was done by using following procedure adopted by Jasmy (2016). Treatment solutions (5 mL) were sprayed separately by topical application with an atomiser on healthy uniform sized second instar larvae collected from established cultures. The treated pre starved larvae were transferred into sterile Petri plates (9 cm) lined with tissue paper. They were provided with fresh bitter gourd leaves, daily. The leaves were kept afresh by winding the petiole with a cotton swab dipped in water. Three replications were maintained with 10 larvae per replication. Larvae sprayed with sterile water served as control. Observations were recorded at 24 h interval on mortality and behavioural changes if any, till they pupated. Larval mortality was calculated using the following formula.

$$\text{Per cent larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

3.1.3.1.2 S. litura

3.1.3.1.2.1 Leaf dip method

Fresh castor leaf discs of uniform diameter (9 cm) were treated as mentioned in para 3.1.3.1.1.1 with similar experimental set up and observations.

3.1.3.1.2.2 Spray method

Uniform sized, healthy second instar larvae selected from established cultures were treated as mentioned in para 3.1.3.1.1.2 and fed with castor leaves with the same experimental set up and observations.

3.1.3.1.3 Coleoptera

3.1.3.1.3.1 H. vigintioctopunctata

3.1.3.1.3.1.1 Leaf dip method

Experiment was laid out with second instar grubs (five day old) and adults fed with brinjal leaf discs and observations were recorded as described in para 3.1.3.1.1.1.

3.1.3.1.3.1.2 Spray method

Uniform sized healthy adults and second instar grubs selected from rearing cultures were treated and kept under observations as described in para 3.1.3.1.1.1.2.

3.1.3.1.3.2 M. viridanus

Screening of grubs for antifeedant effect was excluded from the experiment due to their root feeding nature and the treatments were tested for antifeedant activity as well as mortality to adults.

3.1.3.1.3.2.1 Leaf dip method

Experiment was laid out with healthy adults fed with brinjal leaf discs and observations were recorded as described in para 3.1.3.1.1.1.1.

3.1.3.1.3.2.2 Spray method

Healthy adult weevils selected from laboratory culture were subject to treatment as described in para 3.1.3.1.1.1.2 with a similar experimental set up and observations.

3.1.3.1.5 Hemiptera

3.1.3.1.5.1 A. craccivora

Being a sucking pest, leaf dip method was excluded from the experiment.

3.1.3.1.5.2 Spray method

Healthy second instar nymphs (four day old) were collected from laboratory culture. They were then transferred separately to Petri plates of 9 cm diameter, lined with tissue paper. Each of the treatment solutions (5 mL) was sprayed on nymphs by using an atomiser. The treated insects were fed as mentioned in para 3.1.1.1.3.2. Three replications were maintained with 50 aphids per replication. Observations on mortality were recorded at 24 h interval. Aphids treated with sterile water served as control.

3.1.3.1.5.2 R. pedestris

Leaf dip method was excluded as it is a sucking pest.

3.1.3.1.5.2.1 Spray method

Healthy uniform sized second instar nymphs (five day old) selected from rearing cultures were released into separate plastic troughs. The treatment solutions (five mL) were sprayed on the bugs using an atomiser. Treated bugs were released into plastic troughs (15 x 10 cm) and provided with feed as described in para 3.1.3.5.1 and observations were recorded as mentioned in the same para.

3.1.3.2 Plant Parasitic Nematodes

3.1.3.2.1 M. incognita

3.1.3.2.1.1 Egg mass

The effect of biopolymers on hatching of egg masses were evaluated by adopting the method described by Asif *et al.* (2014). Fresh egg masses were

collected from infested tomato plants mentioned in 3.1.1.2.1. Egg masses were suspended in 5 mL of each of the treatment solutions taken in small glass vials @ 5 egg masses per vial. Egg masses suspended in sterile water served as control. The experiment was replicated thrice. The number of eggs hatched was noted up to four days or till all of them have hatched in the control, whichever is earlier.

3.1.3.2.1.2 J_2 stage

Laboratory screening of juveniles was done based on the method adopted by Khurma and Singh (1997) with slight modification. Freshly hatched second stage juveniles (N =110) in 5 mL sterile water was suspended in each of the treatment solutions of double concentration (taking into account the dilution resulting from five mL sterile water taken for nematode suspension) taken in sterile glass vials. Before adding the treatment solutions, they were filtered through a muslin cloth to avoid debris. Juveniles in 10 mL sterile water served as control. Mortality was observed under a stereo microscope, at 24 h interval up to three days.

3.1.3.2.2 R. reniformis

Being a semi endoparasitic species, screening of eggs was not done. Equal number of nematodes collected from rearing cultures was utilized for the experiment. Pre-adult stage juvenile suspension in five mL sterile water (N=300) was transferred into separate sterile vials with treatment solutions as described in para 3.1.3.2.1.2 and observations were recorded for three days as in the case of *M. incognita*.

3.1.3.2.3 Helicotylenchus sp.

Freshly washed homogeneous adults (N=100) were treated (different stages could not be distinguished) and kept under observation as described in para 3.1.3.2.1.2.

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3.1.3.3 Natural Enemies

3.1.3.3.1 Coccinellids

Uniform aged adults and grubs collected from laboratory culture were released in to 9 cm sterile plastic Petri plates lined with tissue paper. Treatment solutions (5 mL) were sprayed on adults using an atomizer. Beetles sprayed with sterile water served as control. *A. craccivora* colonies were provided as feed. Three replications were maintained with five beetles per replication. Observations were recorded for mortality at 24 h interval.

3.1.3.3.2 Syrphids

Healthy uniform aged maggots (6 - 8 day old) of *I. scutellare* and *X. scutellare* were placed separately in sterilized Petri plates lined with tissue paper. Experimental lay out and observations were as same as that described in 3.1.3.2.1.

3.1.3.3.3 Spiders

Healthy adults of *T. mandibulata* and *T. maxillosa* were transferred into clean plastic covers and were sprayed with each of the treatment solutions using a atomizer. Treated spiders were transferred into plastic troughs provided with aphids as feed. Spiders sprayed with sterile water served as control. Each treatment was replicated thrice with three spiders per replication. Mortality was recorded at 24h interval till the end of the experimental period.

3.2 POT CULTURE STUDIES

Pot culture experiments were conducted (Plate 5) to evaluate the potential of biopolymers selected from laboratory studies, against pests selected based on their positive response, by raising their respective crop plants. One test organism each, representing the Orders Lepidoptera, Coleoptera and Hemiptera were selected for the study. The treatments were evaluated based on the effectiveness

Plate 5. General view of pot culture experiments

in reducing the insect population, incidence of other pests and diseases as well as the growth parameters of crop plant.

3.2.1 D. indica

Experiment was carried out in design CRD with three effective treatments selected from laboratory screening along with an untreated check. The treatments selected were chitin 7%, chitosan 7% and chitosan gel 7%. Each treatment was replicated four times with two plants per replication.

3.2.1.1 Raising Crop

To raise the host plant, bitter gourd seedlings of variety Preethi were raised in grow bags (30 cm diameter) filled with 1:2:1 potting mixture (sand: soil: cowdung). Crop was maintained according to KAU package of practice recommendations (KAU, 2011) excluding plant protection measures.

3.2.1.2 Evaluation of Treatments

3.2.1.2.1 Leaf area damage of D. indica

Leaf area damage was accessed on three, five and seven days after treatment by selecting six leaves from each plant (two each from upper, middle and lower strata). Calculated the per cent of feeding inhibition by using the formula mentioned in para 3.1.3.1.1.1.1.

3.2.1.2.2 Incidence of other pests and diseases

Incidence of *Epilachna septima* Dieke noted during the period of study was assessed by scoring the percentage of leaves damaged, as below. 0 - no damage ; 1 - 1 to 10 per cent damage ; 3 - 11 to 25 per cent damage; 4 - 26 to 50 per cent damage; 5 - 51 to 75 per cent damage ; 6 - >76 per cent damage.

Incidence of viral mosaic was assessed based on the scores specified by Arunachalam (2002). 0 - No symptom; 1 - Minute chlorotic specks/patches on leaf; 2 - Wide area of mosaic symptom on whole leaf without distortion;

3 - Distortion and reduction about 25 per cent of the normal leaf area; 4 - Distortion and reduction about 25 to 75 per cent of the normal leaf area; 5 - Distortion and reduction about more than 75 per cent of the normal leaf area. Percent of disease index was calculated by using following formula.

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves observed x Maximum disease grade}} \times 100$$

3.2.1.2.3 Growth parameters of bitter gourd

Plant height, number of leaves and number of branches per plant were recorded at fortnightly interval and yield per plant at the time of each harvest.

3.2.2 *H. vigintioctopunctata*

Experiment was carried out in design CRD with two effective treatments selected from laboratory study along with an untreated control. The treatments selected were chitosan 7% and chitosan gel 7%. Each treatment was replicated five times with two plants per replication.

3.2.2.1 Raising Crop

Brinjal seedlings of variety Haritha, were raised in grow bags as mentioned in para 3.2.1.1.

3.2.2.2 Evaluation of Treatments

3.2.2.2.1 Population of H. vigintioctopunctata

Pre count and post count of the pests (including both grubs and adults) were taken on third, fifth and seventh day after spraying.

3.2.2.2.2 Incidence of other pests and diseases

Incidence of the leaf weevil *M. viridanus* and *A. gossypii* were assessed by scoring in a 0 to 5 scale. *M. viridanus* leaf damage was scored as follows. No

damage (0) ; 1 to 10 per cent damage (1) ; 11 to 25 per cent damage (2) ; 26 to 50 per cent damage (3) ; 51 to 75 per cent damage (4) and >76 per cent damage (5).

Aphid incidence was scored using modified method of Nagrare *et al.* (2011). The scores were as follows. No aphids (0) ; Scattered appearance of few aphids on the plant (1) ; Severe infestation of aphids on any branches of the plant (2) ; Severe infestation of aphids on more than one branch or half of the plant (3) ; Severe infestation of aphids on whole plant (4).

Fruit borer incidence was scored at the time of harvest, using the following scores suggested by Hautea *et al.* (2004). No damage (0); 0 to 5 per cent of fruits damaged (1) ; 6 to 20 per cent of fruits damaged (2); 21 to 40 per cent of fruits damaged (3); 41 to 60 per cent of fruits damaged (4) and 61 to 100 per cent fruits damaged (5).

No diseases were observed during the study.

3.2.2.2.3 Growth parameters of brinjal

Growth parameters were recorded as mentioned in para 3.2.1.2.3.

3.2.3 A. craccivora

Experiment was carried out in design CRD with four effective treatments selected from laboratory screening along with an untreated check. Each treatment was replicated four times with two plants per replication. The effective treatments selected were chitin 7%, chitosan 7%, chitosan gel 7% and Biorakshak 2%.

3.2.3.1 Raising Crop

Cowpea variety, Bhagyalakshmi were raised in grow bags as mentioned in para 3.2.2.1.

3.2.3.2 Evaluation of Treatments

3.2.3.2.1 Population of A. craccivora

Incidence of aphids on cowpea was recorded commencing from four weeks after planting. Aphid population on the 15 cm long terminal twig with unopened leaves and two opened leaves were taken before and on the third, fifth and seventh day of the treatment. Based on the intensity of infestation these twigs were classified into five classes as follows (Banks, 1954). No aphids (0); Very light infestation - from one aphid to small colony confined to the very youngest leaves of the crown (VL); Light infestation - several colonies present on the stem, not only confined to the uppermost leaves (L) ; Medium - aphids present in large numbers, not in recognisable colonies but diffuse and infesting a large proportion of leaves and stem (M) ; Heavy - aphids present in large numbers very dense, infesting all the leaves and stem, the later usually being black with aphids (H).

Estimation of number of aphids in each class was done by the method suggested by Srikanth (1985). Ten numbers of shoots in each class were collected from field and brought to the laboratory. Each sample shoot was then transferred to a white paper and were gently tapped to dislodge the aphids. The mean number of aphids (all stages) per twig in each class was calculated as follows

Class	Number of aphids per sample										Mean number of aphids per class
	1	2	3	4	5	6	7	8	9	10	
V	28	3	6	2	22	14	30	28	16	19	16.8
L	52	48	36	7	58	80	73	56	64	78	61.2
M	92	86	83	99	123	142	116	138	146	128	115.3
H	206	283	381	386	403	253	306	272	289	356	313.5

3.2.3.2.2 Incidence of other pests and diseases

Red spider mite *Tetranychus* sp. infestation was assessed using the scoring pattern suggested by Kaur *et al.* (2010). No infestation (0); 10 per cent

leaf damage (1); 25 per cent leaf damage (2); 50 per cent leaf damage (3); 75 per ent leaf damage (4); 100 per cent damage (5).

No disease was observed during the study.

3.2.3.2.3 Growth parameters

Observations on growth parameters were recorded as described in para 3.2.1.2.3.

3.2.4 M. incognita

Experiment was carried out in design CRD with three effective treatments selected from laboratory screening along with an untreated check. The treatments selected were chitin 7% (crude), chitosan 7% (crude), chitosan gel 7%. Each treatment was replicated four times with two plants per replication.

3.2.4.1 Raising Crop

Tomato seedlings, variety Anagha were raised in grow bags as mentioned in para 3.2.2.1.

3.2.4.2 Inoculation of Nematodes

Highly infected root samples (two gram) were collected from laboratory culture mentioned in para 3.1.1.2.1 and inoculated near the root zone by making small hole about 6 cm deep and 1.5 cm away from the base of the plant. Equal amount of infected roots were added to all the plants. Holes were closed immediately using moist soil. The plants were given mild irrigation to keep the soil moist.

3.2.4.3 Application of Treatments

The treatments were applied as soil amendments, to the plant base by making 10 - 15 cm deep circular trenches near the root zone without damaging the roots and were closed with moist soil.

3.2.4.4 Evaluation of Treatments

3.2.4.4.1 Population of nematodes

Treatments were evaluated based on the number of nematodes per 200 g soil sample and five gram of root sample at 90 DAT.

3.2.4.4.1.1 Estimation of nematodes from root samples

Nematodes present in 5 g of root samples was estimated by adopting the method described by Brown *et al.* (1985). Five gram each of root was taken from the treatment plants for estimating the population. For this, the root was teased out using a sharp knife and then washed thoroughly under running tap water. It was then chopped into bits of two to three cm length and was subjected to differential staining using acid fuschin stain and plain lactophenol. This was done by boiling the root samples in a hot water bath, with acid fuschin stain taken in a 100 mL beaker and thereafter washing it in tap water. They were then treated with plain lactophenol for 24 h, so as to destain. The samples were then dissected under a stereo microscope and the number of females was counted using a tally counter.

3.2.4.4.1.2 Estimation from soil samples

Soil samples (200 g) were collected from the root zone was processed by the method suggested by Siddiqui *et al.* (2001). Nematodes were extracted by using Cobb's sieving and decanting method followed by modified Baermann's funnel technique. The nematode suspension was made up to 100 mL and an aliquot of five mL was pipette out into a counting dish and were counted under stereomicroscope. This process was repeated for two to three times. The mean number was taken for the statistical analysis.

3.2.4.4.2 Incidence of other pests and diseases

Incidence of American serpentine leaf miner, *Liriomyza trifolii* Burgess was scored by using the following scores (Reji, 2002). No damage (0) ; 1 to 25

per cent leaves show damage symptom (1) ; 26 to 50 per cent damage (2) ; 51 to 75 per cent damage (3) ; >76 percent leaf damage (4).

Cercospora leaf spot disease observed during the study was scored by using the following scores (Oladiran, 1983). No disease (0); 1 to 10 per cent of leaf area infected (1); 11 to 25 per cent of leaf area infected (2); 26 to 50 per cent of leaf area infected (3); 51 to 75 per cent of leaf area infected (4); >76 per cent of leaf area infected (5). Percent of disease index was calculated by using the formula mentioned in para 3.2.1.2.2.

3.2.4.4.3 Growth parameters

Observations were recorded on the number of leaves, number of branches, plant height and yield per plant.

3.3 FIELD EXPERIMENT TO EVALUATE THE EFFICACY OF CHITIN AND CHITOSAN IN MANAGING INSECT AND NEMATODE PESTS

The superior treatments selected from pot culture studies were evaluated for their efficacy in managing the pest population under field conditions (Plate 6). Two experiments were conducted, one each for the insect and nematode pest.

3.3.1 *A. craccivora* in Cowpea

To evaluate the efficacy of chitin and chitosan in the management of *A. craccivora*, an experiment was laid out in RBD with four treatments (chitin 7 %, chitosan 7%, chitosan gel 7%, Dimethoate 0.2 % as chemical check and untreated check) and five replications.

Cowpea was raised as per KAU package of practices (KAU, 2011) excluding the plant protection measures. Unit plot size was 1 m^2 with a spacing of 30 x 15 cm. The treatments were given as foliar spray when 50 per cent of the plants were infested.

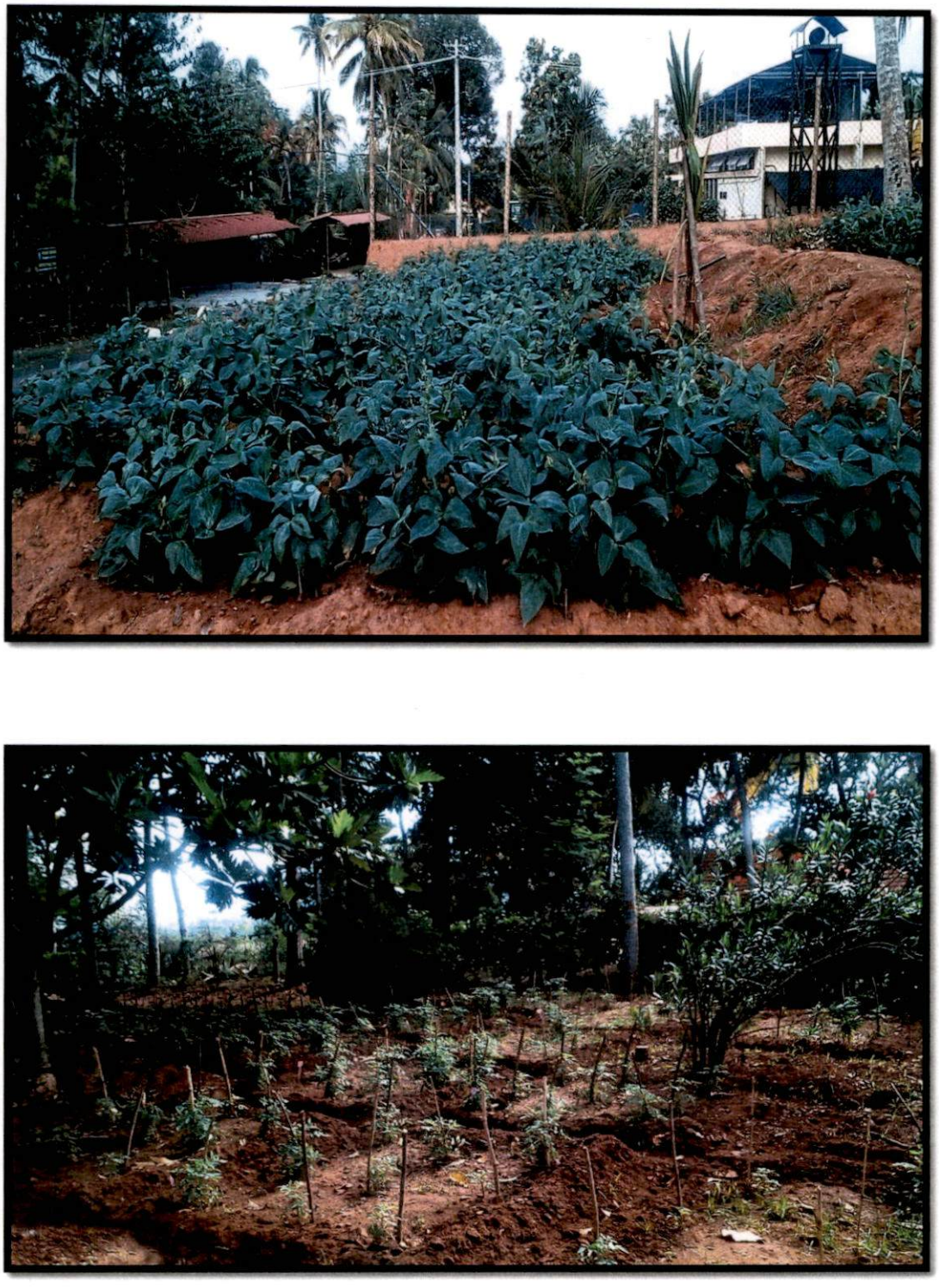

Plate 6. Field experiments on cowpea (top) and tomato (bottom)

3.3.1.1 Assessment of population of A. craccivora

The aphid count was taken following sampling technique mentioned in para 3.2.3.2.1 as O, V, L, M and H.

3.3.1.2 Estimation of Yield

Yield was recorded separately from each treatment during harvest.

3.3.1.3 Assessment of Incidence of Other Pests and Diseases

There was no incidence of other pests and diseases during the assessment period.

3.3.2 *M. incognita* in Tomato

An experiment was laid out in RBD to evaluate the efficacy of chitin and chitosan in the management of *M. incognita*. The treatments evaluated were chitin 7 %, chitosan 7%, Cartap hydrochloride 4G @ 1kg a.i ha^{-1} (chemical check) and an untreated check) with four replications.

Tomato was raised as per KAU package of practices (KAU, 2011) excluding the plant protection measures. Unit plot size was 2m x 2m, with spacing of 60 x 60 cm. The experiment was carried out in an area infested by plant parasitic nematodes. Treatments were applied in soil in shallow circular trenches taken around the root zone, without damaging the roots, 10 days after planting.

The amount of chitin and chitosan required for one plant was calculated based on the fact that an area of one hectare hoards 2.24 $x10^6$ kg of soil. One plant occupies a root zone of 15 x 15 cm which hoards 5.04 kg soil. Therefore, quantity of crude chitin 7%, crude chitosan 7% and chitosan gel 7% was calculated as 35.28 g $plant^{-1}$ and cartap hydrochloride @ 1kg a.i ha^{-1}, 0.55 g $plant^{-1}$.

3.3.2.1 Assessment of Population of M. incognita

The nematode population was assessed based on the soil and root samples taken before and after treatment. Soil samples from 15 cm depth were collected from three spots in each plot. They were pooled and quartered to obtain 200 g. Nematodes were extracted and counted as mentioned in para 3.2.4.4.1.2.

3.3.2.2 Assessment of Incidence of Other Pests and Diseases

Incidence of *L. trifolii* and cercospora leaf spot was scored by the method mentioned in 3.2.4.4.2. Bud necrosis and leaf curl virus incidence was calculated by using the following formula given by Rajasekharam (2010).

$$\text{Disease incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

3.3.2.3 Estimation of Yield

To compare the yield in the different treatments weight of fruits obtained from each plot was recorded.

3.4 COST ANALYSIS

3.4.1 Benefit Cost Ratio

Cost analysis was calculated in terms of benefit cost (B: C) ratio by using following formula

$$\text{B: C ratio} = \frac{\text{Gross income (Rs ha}^{-1}\text{)}}{\text{Cost of cultivation (Rs ha}^{-1}\text{)}}$$

3.5 STATISTICAL ANALYSIS

Data from laboratory experiments on mortality and percentage of egg hatching were analysed by one way analysis after arc sin transformation. Population of insect pests and nematodes were analysed by one way analysis after

square root transformation except aphid population in pot culture (logarithmic transformation). All the data were analysed in WASP 1.0 software.

Results

4. RESULTS

The results of the investigation entitled "Potential of the natural bio polymers, chitin and chitosan in pest management" carried out during 2015 - 17 in the Department of Agricultural Entomology, College of Agriculture, Vellayani are presented below.

4.1 EFFECT OF THE NATURAL BIOPOLYMERS, CHITIN AND CHITOSAN ON INSECT PESTS, PLANT PARASITIC NEMATODES AND NATURAL ENEMIES UNDER LABORATOTY CONDITIONS

The different formulations evaluated were chitin (3, 5 and 7%), chitosan (3, 5 and 7%) chitosan gel (3, 5 and 7%), chitin based bioformulation, Biorakshak 2% and chitosan based commercial formulation, Bioboost 2%. Three different Orders, Lepidoptera (*Diaphania indica* Saunders. and *Spodoptera litura* F.), Coleoptera (*Henosepilachna vigintioctopunctata* F. and *Myllocerus viridanus* Schoenherr), and Hemiptera (*Aphis craccivora* Koch and *Riptortus pedestris* F.), three plant parasitic nematodes (*Meloidogyne incognita* (Kofoid and White) Chitwood, *Rotylenchulus reniformis* Linford and Oliveira and *Helicotylenchus sp*) and three group of natural enemies *viz.*, the coccinellids (*Chilomenes sexmaculata* F. and *Coccinella transversalis* F.) the syrphids (*Ischiodon scutellare* F. and *Xanthogramma scutellare* Thorell) and the spiders (*Tetragnatha mandibulata* Walckenaer and *Tetragnatha maxillosa* Thorell). The treatments were assessed based on the behavioural changes, feeding inhibition and mortality caused to the test organisms.

4.1.1 Insect Pests

4.1.1.1 Lepidoptera

4.1.1.1.1 Diaphania indica Saunders

4.1.1.1.1.1 Feeding activity

Larvae fed with treated leaves were inactive with less feeding activity.

The effect of chitin and chitosan on feeding inhibition of *D. indica* is presented in Table 1. One day after treatment, highest inhibition of 43.42 per cent was recorded in chitosan 7% (Plate 7) which was closely followed by the treatment, chitosan gel 7% (42.85 per cent). The next level of inhibition was noted in leaves treated with chitosan gel 5% (38.88 per cent), whereas with chitin 7% the inhibition was significantly lower, 25.4 per cent. The inhibition noted with chitosan 5% was still lower (23.28 per cent) but significantly higher than the inhibition level noted with chitin 5% and 3% which were statistically on par (11.79 and 11.50 respectively). Chitosan 3% (5.84 per cent) and Biorakshak 2% (6.71 per cent), which were on par with each other recorded the least level of inhibition.

Second day also, chitosan 7% recorded the highest inhibition of 38.57 per cent, followed by chitosan 5% (35 per cent) and chitosan gel 7% (32.73 per cent), which were on parity. The third level of inhibition was noted in leaves treated with chitin 7% (32.14 per cent), which was followed by chitosan gel 5% (21.15 per cent). The inhibition noted with chitin 5%, chitosan gel 3% and Bioboost 2% were statistically indifferent (14.63, 16.0 and 12.15 respectively). Biorakshak 2% (7.80 per cent) was inferior to all the above treatments but superior to chitosan 3% (16.10 per cent), chitosan gel 3% (16.0 per cent) and chitin 3% (6.60 per cent), which were on par with each other.

On the third day, chitosan gel 7% recorded maximum feeding inhibition of 17.6 per cent, followed by chitosan 7% (14.2 per cent). Third level of inhibition was noted with chitin 7% (12.3 per cent), which was statistically superior to

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Chitosan 7%

Chitosan gel 7%

Chitin 7%

Control

Plate 7. Feeding inhibition of chitin and chitosan on *D. indica* larvae

(1 DAT)

Table 1. Effect of chitin and chitosan on feeding activity of larvae of *D. indica* under laboratory conditions

Sl.no	Treatments	Feeding inhibition (%) at 24 h interval			
		Day 1	Day 2	Day 3	Day 4
1	Chitin 3%	11.50^{de}	6.60^{d}	0.94^{e}	2.12^{de}
2	Chitin 5%	11.79^{de}	14.63^{bcd}	4.97^{de}	3.40^{cd}
3	Chitin 7%	25.4^{bcd}	32.14^{abc}	12.3^{bc}	6.13^{b}
4	Chitosan 3%	5.84^{e}	16.10^{d}	0.31^{e}	0.90^{e}
5	Chitosan 5%	23.28^{cde}	35.0^{ab}	1.26^{e}	2.13^{de}
6	Chitosan 7%	43.42^{a}	38.57^{a}	14.2^{ab}	13.90^{a}
7	Chitosan gel 3%	20.17^{de}	16.0^{bcd}	0.63^{e}	2.12^{de}
8	Chitosan gel 5%	38.88^{abc}	21.15^{abcd}	8.86^{cd}	3.71^{cd}
9	Chitosan gel 7%	42.85^{ab}	32.73^{ab}	17.6^{a}	4.39^{c}
10	Bioboost 2%	11.79^{de}	12.15^{bcd}	8.42^{cd}	3.06^{cd}
11	Biorakshak 2%	6.71^{e}	7.80^{cd}	7.74^{cd}	3.65^{cd}
	CD (0.05)	17.862	20.784	4.844	1.656

Mean of three replications

Table 2. Effect of chitin and chitosan on feeding activity of larvae of *S. litura* under laboratory conditions

Sl.no	Treatments	Feeding inhibition (%) at 24 h interval			
		Day 1	Day 2	Day 3	Day 4
1	Chitin 3%	0.08	0	0	0
2	Chitin 5%	0	0.29	0	0
3	Chitin 7%	0.09	0.26	0	0
4	Chitosan 3%	0.37	0.08	0.03	0.03
5	Chitosan 5%	0.21	0.34	0.03	0.05
6	Chitosan 7%	0.55	0.52	0.34	0.08
7	Chitosan gel 3%	0.27	0.14	0.05	0
8	Chitosan gel 5%	0.43	0.28	0.08	0.03
9	Chitosan gel 7%	0.70	0.44	0.30	0.08
10	Bioboost 2%	0.20	0.16	0.03	0.03
11	Biorakshak 2%	0	0.17	0	0

Mean of three replications

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chitosan gel 5%, Bioboost 2% and Biorakshak 2% (8.86 per cent, 8.42 per cent and 7.74 per cent, respectively). The inhibition was very less with chitin 5% (4.97 per cent) and negligible in chitosan 3% (0.31 per cent), chitosan gel 3% (0.63 per cent), chitin 3% (0.94 per cent) and chitosan 5% (1.26 per cent).

Maximum inhibition recorded on fourth day was with chitosan 7% (13.90 per cent) which is superior to over all the treatments. This was followed by chitin 7% (6.13 per cent), which was statistically higher to chitosan gel 7% (4.39 per cent), chitin 5% (3.40 per cent), Biorakshak 2% (3.65 per cent) and Bioboost 2% (3.06 per cent). Negligible inhibition was noted with chitin 3%, chitosan gel 3% (2.12 per cent each) and chitosan 3% (2.13 per cent).

None of the treated insects died earlier than the death observed in untreated insects.

4.1.1.1.2 Spodoptera litura F.

Larvae treated with various formulations did not exhibit any behavioural changes except for less amount of feeding inhibition. Table 2 depicts the effect of various formulations of chitin and chitosan on feeding activity of *S. litura* larvae. None of the treatments showed neither significant inhibitory effects on the feeding activity nor mortality to the caterpillars.

4.1.1.2 Coleoptera

4.1.1.2.1 Henosepilachna vigintioctopunctata F.

4.1.1.2.1 Feeding activity

Grubs were found to be less active when fed with treated leaves. As revealed in Table 3, the feeding inhibition expressed by *H. vigintioctopunctata* grubs is meager, the highest inhibition being 5.01 per cent, observed with chitosan gel 7%. The least inhibition noted was 2.03 per cent with chitin 3%. At the end of the experimental period (Day 4), the inhibitory levels ranged from a maximum

Table 3. Effect of chitin and chitosan on feeding activity of *H. vigintioctopunctata* under laboratory conditions

Sl.no	Treatments	Feeding inhibition (%) at 24 h interval							
		Day 1		Day 2		Day 3		Day 4	
		Grubs	Adults	Grubs	Adults	Grubs	Adults	Grubs	Adults
1	Chitin 3%	2.03^{c}	0.93^{de}	1.8^{cd}	0.16^{d}	1.20^{bc}	0.09^{c}	1.71^{d}	3.11^{bcd}
2	Chitin 5%	2.25^{bc}	1.33^{de}	3.3^{ab}	0.71^{bcd}	1.60^{b}	0.66^{bc}	2.67^{bcd}	3.71^{abc}
3	Chitin 7%	3.98^{ab}	1.56^{bcd}	4.3^{a}	1.58^{bc}	3.67^{a}	1.70^{ab}	4.17^{a}	4.65^{ab}
4	Chitosan 3%	2.57^{bc}	0.96^{e}	2.1^{c}	0.79^{bcd}	1.85^{b}	0.48^{bc}	2.13^{cd}	0.47^{e}
5	Chitosan 5%	3.45^{abc}	1.51^{cde}	2.8^{bc}	1.43^{bcd}	2.7^{a}	0.37^{bc}	2.59^{bcd}	1.74^{cde}
6	Chitosan 7%	3.23^{abc}	5.37^{a}	4.2^{a}	5.4^{a}	3.15^{a}	2.85^{a}	3.6^{bc}	5.77^{a}
7	Chitosan gel 3%	2.20^{bc}	0.77^{de}	0.9^{d}	0.36^{cd}	0.53^{c}	0.67^{bc}	1.54^{d}	0.64^{e}
8	Chitosan gel 5%	5.01^{a}	1.7^{abc}	2.2^{c}	0.22^{d}	1.37^{bc}	0.90^{bc}	2.59^{cd}	0.95^{de}
9	Chitosan gel 7%	3.82^{abc}	1.92^{ab}	4.3^{a}	1.75^{b}	2.86^{a}	1.04^{bc}	3.90^{b}	3.86^{abc}
10	Bioboost 2%	2.37^{bc}	0.13^{de}	2.5^{bc}	0.19^{d}	1.72^{b}	0.72^{bc}	2.52^{bcd}	0.83^{e}
11	Biorakshak 2%	2.25^{bc}	0.073^{e}	2.2^{c}	0.2^{d}	1.52^{bc}	0.11^{c}	2.41^{bcd}	1.83^{cde}
	CD(0.05)	1.834	17.862	1.025	20.784	1.006	1.353	1.575	2.172

Mean of three replications

of 4.17 (Chitin 7%), to a minimum of 1.54 per cent observed with chitosan gel 3% (1.54 per cent).

The inhibition was very less in adults too, highest being 5.37 per cent with chitosan 7% and lowest with chitosan 3% and Biorakshak 2% (0.96 and 0.73 per cent respectively), after one day. There was no significant change in the trend, at the end of fourth day, the range being 0.33 to 5.77 per cent.

4.1.1.2.1 Mortality of H. vigintioctopunctata

The effect of treatments on mortality of *H. vigintioctopunctata* is presented in Table 4. There was no appreciable mortality of grubs one day after treatment.

After two days, 20 per cent mortality was noted in chitosan 5% and 7% and chitosan gel 7%. Bioboost 2% and Biorakshak 2% ranked next with 13.33 and 6.66 per cent respectively, which were statistically on par. There was no mortality in any other treatments.

Three days after treatment, chitosan 7% and chitosan gel 7% had similar effect, with 40 per cent mortality for each, followed by chitosan 5% (33.33 per cent). Mortality observed in Bioboost 2% (26.66 per cent), chitin 5%, chitin 7%, chitosan 3% and Biorakshak 2% (20 per cent each) were statistically same. Chitin 3 % caused least mortality of 13.33 per cent which was inferior to above treatments and superior to chitosan gel 3%, chitosan gel 5% and control (6.66 per cent each).

On the fourth day, highest mortality was noted with chitosan gel 7% (60 per cent) followed by chitosan 7% (53.33 per cent). Chitosan 5% and Bioboost 2% resulted in 40 and 46.66 per cent mortality, respectively which were statistically similar. The corresponding mortality in chitin 7% was 33.33 per cent, which ranked third. Chitosan 3% killed 26.66 per cent grubs and was superior to chitin 3%, chitin 5% and Biorakshak 2%, which resulted in 20 per cent mortality,

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Table 4. Effect of chitin and chitosan on mortality of *H. vigintioctopunctata* grubs under laboratory conditions

Sl. no	Treatments	Cumulative mortality (%) at 24 h interval						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Chitin 3%	0	0 (1.28)^{c}	13.33 (18.13)^{bc}	20 (26.56)^{cde}	20 (26.56)^{cde}	26.66 (30.78)^{c}	26.66 (30.78)^{fg}
2	Chitin 5%	0	0 (1.28)^{c}	20 (26.56)^{abc}	20 (26.56)^{cde}	26.66 (30.78)^{cd}	40 (39.23)^{c}	40 (39.23)^{ef}
3	Chitin 7%	0	0 (1.28)^{c}	20 (26.56)^{abc}	33.33 (34.63)^{abcd}	46.66 (43.07)^{bc}	73.33 (63.79)^{ab}	73.33 (63.79)^{bcd}
4	Chitosan 3%	0	0 (1.28)^{c}	20 (26.56)^{abc}	26.66 (30.78)^{bcd}	40 (35.00)^{cd}	53.33 (46.92)^{bc}	60 (51.14)^{cdef}
5	Chitosan 5%	0	20 (26.56)^{a}	33.33 (35.00)^{ab}	40 (39.23)^{abc}	60 (39.23)^{bc}	73.33 (59.21)^{b}	86.66 (71.86)^{abc}
6	Chitosan 7%	0	20 (26.56)^{a}	40 (39.23)^{a}	53.33 (46.92)^{ab}	66.66 (54.99)^{ab}	80 (63.43)^{ab}	86.66 (71.86)^{abc}
7	Chitosan gel 3%	0	0 (1.28)^{c}	6.66 (9.70)^{c}	6.66 (9.70)^{e}	20 (30.78)^{cd}	33.33 (35.00)^{c}	46.66 (46.92)^{def}
8	Chitosan gel 5%	0	0 (1.28)^{c}	6.66 (9.70)^{c}	13.33 (18.13)^{de}	13.33 (18.13)^{de}	53.33 (46.92)^{bc}	66.66 (54.99)^{cde}
9	Chitosan gel 7%	6.66	20 (22.35)^{ab}	40 (38.85)^{a}	60 (51.14)^{a}	80 (67.64)^{a}	93.33 (80.29)^{a}	100 (88.71)^{a}
10	Bioboost 2%	6.66	13.33 (18.13)^{ab}	26.66 (26.58)^{abc}	46.66 (42.70)^{abc}	66.66 (54.99)^{ab}	80 (63.43)^{ab}	93.33 (80.29)^{ab}
11	Biorakshak 2%	6.66	6.66 (9.70)^{bc}	20 (26.56)^{abc}	20 (26.56)^{cde}	26.67 (30.78)^{cd}	33.33 (34.63)^{c}	33.33 (34.63)^{ef}
12	Control	0	0 (1.28)^{c}	6.66 (9.70)^{c}	6.66 (9.70)^{e}	6.66 (9.70)^{e}	6.66 (9.70)^{d}	6.66 (9.70)^{g}
	CD(0.05)	NA	13.757	19.044	17.367	18.666	18.733	21.520

Figures in parentheses are angular transformed values NA - Not analysed
Mean of three replications

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each. Chitosan gel 5% recorded 13.33 per cent which was inferior to above treatments and superior to chitosan gel 3% (6.66 per cent), which is on par with control.

On the fifth day, the chitosan gel 7% caused maximum mortality of 80 per cent, which is superior over all the treatments. The treatments, chitosan 7% and Bioboost 2% caused 66.66 per cent (each) mortality, followed by chitosan 5% and chitin 7% which recorded 60 and 46.66 per cent respectively, and were on parity. The corresponding mortality in chitosan 3%, Biorakshak 2%, chitin 5% and chitosan gel 3% was 40, 26.67, 20 and 26.66 per cent respectively, which were statistically on par. The least mortality of 20 per cent was noted in chitin 3%, followed by that observed in chitosan gel 5% (13.33 per cent).

The highest mortality of 93.33 per cent was recorded in chitosan gel 7% on sixth day, which is superior to other treatments. The mortality recorded in Bioboost 2%, chitosan 7% (80 per cent) and chitin 7% (73.33 per cent) was statistically same. This was followed by chitosan 3% and chitosan gel 5% which recorded 53.33 per cent mortality, each. The least mortality was noted in chitin 5%, chitosan gel 3%, Biorakshak 2%, chitin 3% and control (40, 33.33, 33.33 and 26.66 per cent respectively), which were statistically on par.

Hundred per cent mortality was noted with chitosan gel 7% on seventh day which is superior over other treatments. The corresponding mortality in Bioboost 2% was 93.33 per cent. This followed by 86.66 per cent of mortality was recorded in chitosan 5% and chitosan 7%. At this time chitin 7% caused 73.33 per cent mortality and chitosan gel 5% resulted in 66.66 per cent mortality. Sixty per cent mortality was caused by chitosan 3%, and chitosan gel 3% recorded 46.66 per cent. The mortality observed in chitin 5% and Biorakshak 2% was 40 and 33.33 per cent respectively, which were statistically on parity. Significantly least mortality was noted in chitin 3% (26.66 per cent).

At the end of the experiment negligible mortality was recorded in control.

4.1.1.2.2 Myllocerus viridanus Schoenherr

Feeding inhibition exhibited by *M. viridanus* adults is presented in Table 5. The maximum inhibition noted was only 3.32 per cent with chitosan gel 7%. None of the treatments recorded considerable level of mortality throughout the experimental period, though 7% formulations were found to be superior to other.

4.1.1.3 Hemiptera

4.1.1.3.1 Aphis craccivora Koch

The mortality of *A. craccivora* treated with chitin and chitosan is compiled in Table 6. The aphids movement get reduce and become inactive after treatment. There was no significant difference in mortality after one day.

After two days, the treatments varied significantly among them. Maximum mortality of 61.66 per cent was recorded in chitin 7%, closely followed by 60 per cent mortality observed with Biorakshak 2%. Chitin 5% ranked third with 55 per cent mortality. All other treatments resulted in less than 50 per cent mortality. Chitosan 3% and 7% caused 46.66 per cent and 45 per cent mortality respectively, but they were statistically dissimilar. Mortality observed with chitin 3% was 43.33 per cent while, chitosan 5%, chitosan gel 7% and Bioboost 2% were similar in their effect (41.66 per cent). Very less mortality was recorded in chitosan gel 5% (35 per cent) followed by chitosan gel 3% (28.33 per cent). In the control group, the mortality was 2.5 per cent.

Third day after treatment, highest mortality was recorded with chitin 7% (81.66 per cent) which was statistically superior to all other treatments. The mortality recorded in Biorakshak 2%, chitosan 7% and Bioboost 2% ranked next with 76.66, 75 and 71.66 per cent mortality, respectively. The corresponding mortality in chitosan 5% was 70 per cent and that with chitosan gel 7% was 68.3 per cent which were statistically different. Chitin 5%, chitosan 3%, and chitosan gel 5% caused mortality to the tune of 63.33 to 65 per cent, which were on par.

Table 5. Effect of chitin and chitosan on feeding activity of *M. viridanus* adults under laboratory conditions

Sl.no	Treatments	Feeding inhibition (%) at 24 h interval				
		Day 1	Day 2	Day 3	Day 4	Day 5
1	Chitin 3%	0.95^{de}	1.25	1.96^{c}	3.21^{ab}	2.06^{bc}
2	Chitin 5%	1.25^{cde}	1.58	2.22^{bc}	3.44^{a}	2.33^{abc}
3	Chitin 7%	1.36^{cde}	1.29	2.72^{abc}	3.61^{a}	2.43^{abc}
4	Chitosan 3%	1.76^{bcd}	1.14	3.26^{a}	2.04^{bcd}	1.40^{c}
5	Chitosan 5%	2.14^{abcd}	1.92	3.23^{a}	2.33^{abcd}	1.59^{c}
6	Chitosan 7%	2.45^{abc}	2.29	3.42^{a}	2.40^{abc}	2.86^{ab}
7	Chitosan gel 3%	2.52^{abc}	0.59	0.88^{d}	0.97^{de}	2.11^{abc}
8	Chitosan gel 5%	2.76^{ab}	1.22	1.89^{c}	0.58^{e}	2.97^{ab}
9	Chitosan gel 7%	3.32^{a}	2.21	2.94^{ab}	1.04^{cde}	3.22^{a}
10	Bioboost 2%	0.34^{e}	0.64	2.68^{abc}	0.50^{e}	1.69^{c}
11	Biorakshak 2%	1.42^{bcde}	1.54	2.69^{abc}	0.98^{de}	1.61^{c}
	CD(0.05)	1.384	NS	0.954	1.382	1.152

Mean of three replications NS - Non significant

Table 6. Effect of chitin and chitosan on *A. craccivora* nymphs under laboratory conditions

Sl .no	Treatments	Cumulative mortality (%) at 24 h interval				
		Day 1	Day 2	Day 3	Day 4	Day 5
1	Chitin 3%	1.66 (4.73)	43.33 $(41.16)^{bcde}$	53.33 $(46.91)^{d}$	81.66 $(65.0)^{bc}$	88.33 $(70.69)^{bc}$
2	Chitin 5%	1.66 (4.73)	55 $(47.87)^{abc}$	65 $(53.92)^{bcd}$	86.66 $(68.85)^{ab}$	91.66 $(73.79)^{bc}$
3	Chitin 7%	5 (13.90)	61.66 $(51.75)^{a}$	81.66 $(65.0)^{a}$	91.66 $(73.79)^{a}$	100 $(89.35)^{a}$
4	Chitosan 3%	0 (0.645)	46.66 $(43.08)^{abcd}$	65 $(53.76)^{bcd}$	80 $(63.54)^{bc}$	91.66 $(67.40)^{bc}$
5	Chitosan 5%	6.66 (12.11)	41.66 $(40.17)^{cde}$	70 $(56.83)^{abc}$	78.33 $(62.47)^{bc}$	83.33 $(68.85)^{bc}$
6	Chitosan 7%	10 (18.04)	45 $(42.09)^{abcde}$	75 $(60.07)^{ab}$	80 $(63.54)^{bc}$	90 $(70.11)^{bc}$
7	Chitosan gel 3%	8.33 (16.20)	28.33 $(32.14)^{e}$	56.66 $(48.92)^{cd}$	73.33 $(59.05)^{c}$	80 $(71.95)^{bc}$
8	Chitosan gel 5%	8.33 (13.37)	35 $(36.23)^{de}$	63.33 $(52.79)^{bcd}$	78.33 $(62.28)^{bc}$	86.66 $(63.92)^{c}$
9	Chitosan gel 7%	10 (17.46)	41.66 $(40.0)^{cde}$	68.33 $(55.97)^{bc}$	80 $(63.92)^{bc}$	90 $(74.78)^{bc}$
10	Bioboost 2%	8.33 (13.37)	41.66 $(40.0)^{cde}$	71.66 $(57.98)^{ab}$	81.66 $(65.0)^{bc}$	91.66 $(73.40)^{bc}$
11	Biorakshak 2%	6.66 (12.11)	60 $(50.85)^{ab}$	76.66 $(61.14)^{ab}$	83.33 $(65.95)^{abc}$	91.66 $(76.04)^{b}$
12	Control	2.5 (9.09)	2.5 $(20.69)^{f}$	15 $(22.78)^{e}$	15 $(22.78)^{d}$	16 $(22.78)^{d}$
	CD(0.05)	NS	10.11	8.465	7.942	11.17

Figures in parentheses are angular transformed values

Mean of three replications NS - Non significant

Chitosan gel 3%, ranked sixth with 56.66 per cent mortality followed by chitin 3% that resulted in 53.33 per cent death. The corresponding mortality in control was 15 per cent.

On the fourth day also, chitin 7% ranked first, with 91.66 per cent death rate. Chitin 5% recorded 86.66 per cent mortality, which was followed by Biorakshak 2% (83.33 per cent). The mortality noted with chitin 3%, Bioboost 2% (81.66 per cent each), chitosan 3%, chitosan 7%, chitosan gel 7% (80 per cent each) and chitosan 5% (78.33 per cent) were statistically similar. Chitosan gel 3% recorded 73.33 per cent death, while in control it was 15 per cent.

At the end of the experimental period (fifth day), all the treatment resulted more than 80 per cent mortality and it was chitin 7% that stood superior with 100 per cent mortality. Chitin 5%, chitosan 3%, Biorakshak 2% and Bioboost 2% ranked second with 91.66 per cent mortality. Mortality caused by chitin 5%, chitosan 3%, Bioboost 2% (91.66 per cent each), chitosan 7%, chitosan gel 7% (90 per cent each), chitosan 5% (88.33 per cent), chitosan gel 5% (86.66 per cent) and chitosan 5% (83.33 per cent) were statistically similar. The corresponding mortality in chitosan gel 3% was 80 per cent. At the end of the experiment, 16 per cent mortality was recorded in control.

4.1.1.3.2 Riptortus pedestris F.

Neither mortality nor behavioural changes were observed after application of treatments.

4.1.2 Plant Parasitic Nematodes

4.1.2.1 Meloidogyne incognita (Kofoid and White) Chitwood

4.1.2.1.1 Effect on Eggs

M. incognita eggs treated with various formulations of chitin and chitosan exhibited difference in the hatchability (Table 7). One day after treatment, none

Table 7. Effect of chitin and chitosan on hatchability of *M. incognita* under laboratory conditions

Sl. no	Treatments	Egg hatching (%) at 24 h interval			
		Day 1	Day 2	Day 3	Day 4
1	Chitin 3%	20 (26.56)^{abc}	46.66 (43.07)^{abc}	60 (50.76)^{b}	80 (63.43)^{cde}
2	Chitin 5%	26.66 (30.78)^{ab}	46.66 (43.07)^{abc}	53.33 (46.92)^{bc}	60 (50.76)^{efg}
3	Chitin 7%	13.33 (18.13)^{bcd}	26.66 (30.78)^{cd}	33.33 (35.00)^{cde}	46.66 (43.07)^{fg}
4	Chitosan 3%	26.66 (30.78)^{ab}	40 (39.23)^{bc}	53.33 (46.92)^{bc}	60 (50.76)^{efg}
5	Chitosan 5%	6.66 (9.70)^{cd}	13.33 (18.13)^{d}	26.66 (30.78)^{de}	33.33 (35.03)^{g}
6	Chitosan 7%	0 (1.28)^{d}	13.33 (18.13)^{d}	20 (26.56)^{e}	33.33 (35.03)^{g}
7	Chitosan gel 3%	53.33 (46.92)^{a}	60 (50.76)^{ab}	66.66 (54.99)^{b}	93.33 (80.29)^{ab}
8	Chitosan gel 5%	26.66 (26.58)^{abc}	46.66 (43.07)^{abc}	60 (51.14)^{b}	80 (76.06)^{abc}
9	Chitosan gel 7%	33.33 (30.42)^{abc}	40 (38.85)^{bc}	53.33 (46.92)^{bc}	73.33 (59.21)^{def}
10	Bioboost 2%	33.33 (35.0)^{ab}	40 (39.23)^{bc}	46.66 (43.07)^{bcd}	66.66 (59.21)^{def}
11	Biorakshak 2%	46.66 (43.07)^{a}	53.33 (46.92)^{ab}	60 (50.76)^{b}	86.66 (71.86)^{bcd}
12	Control	53.33 (46.92)^{a}	66.66 (54.99)^{a}	86.66 (71.86)^{a}	100 (88.71)a
	CD(0.05)	21.035	14.241	12.848	16.606

Figures in parentheses are angular transformed values

Mean of three replications

of the eggs were hatched in chitosan 7%, showing the superiority of the treatment. The next effective treatment was chitosan 5% where there was 6.66 per cent hatching. The hatchability recorded with chitin 7% ranked third (13.33 per cent), which was significantly superior to chitin 3% (20 per cent) which was statistically on par with chitin 5%, chitosan 3% and chitosan gel 5% (26.66 per cent each). Lowest inhibition of hatching was noted in eggs treated with chitosan gel 7%, and Bioboost 2% (33. 33 per cent each). The least effective treatment was Biorakshak 2% (46.66 per cent) which was on par with control (53.33 per cent).

On second day maximum inhibition on hatching was observed in eggs treated with chitosan 7% and 5% (13.33 per cent), followed by that observed in chitin 7% (26.66 per cent). The effect of chitosan gel 7%, chitosan 3% and Bioboost 2% ranked third in terms of inhibition to hatching (40 per cent each). Negligible effect was noted with chitin 3%, 5% and chitosan gel 5% (46.66 per cent each). Biorakshak 2% (53.33 per cent) and chitosan gel 3% (60 per cent) was least inhibitory and were on par. The number of eggs hatched in control was 66.66 per cent.

On the third day also chitosan 7% was found to be the most effective treatment with least number of hatched eggs (20 per cent), followed by chitosan 5% in which 26.66 per cent eggs hatched. The inhibitory effect of chitin 7% ranked third (33.33 per cent) and was superior to Bioboost 2% (46.66 per cent). The inhibition was very less in chitin 5%, chitosan 3% and chitosan gel 7% (53.33 per cent each). Negligible level of inhibition was noted in eggs treated with chitin 3%, chitosan gel 5% and Biorakshak 2% (60 per cent each). The maximum hatching percentage noted in control was 86.66 per cent.

By fourth day, maximum inhibition of hatching of eggs was noted with chitosan 7% and 5% (33.33 per cent each) followed by chitin 7% (46.66 per cent). Chitin 5% and chitosan 3% were statistically on par with a hatching percentage of 60. This was followed by the effect of chitosan gel 7% and Bioboost 2% which recorded 66.66 and 73.33 per cent hatching respectively. Considerably low

inhibition was observed in chitin 3% followed by Biorakshak 2% (80 and 86.66 per cent respectively). Maximum percentage of hatching was noted among treatments with chitosan gel 3% (93.33 per cent). At the end of the day all the eggs hatched in control.

4.1.2.1.2 Effect on J_2

Table 8 reveals the efficacy of the treatments in causing mortality of juveniles (J_2). A day after treatment, chitosan 7% was found to be superior to other treatments in causing highest mortality of 90 per cent. This was closely followed by chitin 7% and chitosan gel 7% which recorded 86.06 and 85.75 per cent mortality respectively which were on parity. The corresponding mortality with chitosan gel 5% was 81.51 per cent which was statistically superior to chitosan 3, 5 and chitosan gel 3% (78.33, 78.18 and 76.96 respectively). Chitin 5% (73.33 per cent) was superior to chitin 3%, Bioboost 2% (48.98 per cent) and Biorakshak 2% (50.43 per cent) which were statistically similar. The mortality in control was 4.75 per cent.

Hundred per cent mortality was noted with chitin 7%, chitosan 5%, 7% and chitosan gel 7% on the second day which were on par with chitosan 3% and Bioboost 2% (99.39 per cent each). Chitosan gel 5% caused 95.75 per cent mortality of J_2 whereas chitin 5% and Biorakshak 2% recorded 87.27 and 89.69 per cent mortality respectively. The least effect was noted with chitin 3% (72.12 per cent).

All the treatments were found to be equally effective on the third day, causing 95.75 to 100 per cent mortality, while that observed with control was 18.18 per cent.

4.1.2.2 Rotylenchulus reniformis Linford and Oliveira

Effect on pre adult females alone was evaluated, as they are sedentary semi endoparasites (Table 9).

Table 8. Effect of chitin and chitosan on mortality of *M. incognita* juveniles under laboratory conditions

Sl. no	Treatments	Cumulative mortality (%) at 24 h interval		
		Day 1	Day 2	Day 3
1	Chitin 3%	56.96 (49.02)^{e}	72.12 (58.13)^{e}	96.36 (83.38)^{a}
2	Chitin 5%	73.33 (59.09)^{d}	89.69 (71.29)^{d}	99.39 (87.23)^{a}
3	Chitin 7%	86.06 (68.10)^{ab}	100 (89.72)^{a}	100 (89.72)^{a}
4	Chitosan 3%	78.33 (62.40)^{cd}	99.39 (87.23)^{ab}	100 (89.72)^{a}
5	Chitosan 5%	78.18 (62.162)^{cd}	100 (89.72)^{a}	100 (89.72)^{a}
6	Chitosan 7%	90.00 (71.57)^{a}	100 (89.72)^{a}	100 (89.72)^{a}
7	Chitosan gel 3%	76.96 (61.32)^{cd}	93.63 (76.52)^{cd}	96.06 (83.11)^{a}
8	Chitosan gel 5%	81.51 (64.63)^{bc}	95.75 (80.89)^{bc}	95.75 (80.89)^{a}
9	Chitosan gel 7%	85.75 (67.82)^{ab}	100 (89.72)^{a}	100 (89.72)^{a}
10	Bioboost 2%	58.78 (48.98)^{e}	99.39 (87.23)^{ab}	100 (89.72)^{a}
11	Biorakshak 2%	59.39 (50.43)^{e}	87.27 (69.44)^{d}	99.39 (87.23)^{a}
12	Control	4.54 (6.98)^{f}	13.63 (12.26)^{f}	18.18 (14.24)^{b}
	CD(0.05)	4.758	7.083	9.498

Figures in parentheses are angular transformed values

Mean of three replications

Table 9. Effect of chitin and chitosan on mortality of *R. reniformis* under laboratory conditions

Sl.no	Treatments	Cumulative mortality (%) at 24 h interval		
		Day 1	Day 2	Day 3
1	Chitin 3%	48.85(44.33)^{c}	59.47(50.55)^{b}	63.85(53.29)^{bcd}
2	Chitin 5%	49.0(44.51)^{c}	64.68(53.62)^{ab}	74.16(59.46)^{ab}
3	Chitin 7%	66.87(54.93)^{a}	71.6 (57.47)^{a}	78.54 (62.60)^{a}
4	Chitosan 3%	38.75(38.45)^{d}	46.7 (43.14)^{c}	55.72 (48.29)^{de}
5	Chitosan 5%	50.41(45.23)^{bc}	57.29(49.19)^{b}	62.91(52.50)^{cd}
6	Chitosan 7%	57.91(49.55)^{b}	62.18(52.05)^{ab}	66.97(54.95)^{bc}
7	Chitosan gel 3%	13.54(21.57)^{f}	15.52(23.18)^{e}	37.18 (37.50)^{g}
8	Chitosan gel 5%	18.12(25.17)^{f}	21.56(27.60)^{e}	43.43 (41.21)^{fg}
9	Chitosan gel 7%	19.16(26.26)^{ef}	20.83(27.15)^{e}	50.62(45.35) ^{ef}
10	Bioboost 2%	35.62(36.64)^{d}	41.25(39.95)^{cd}	46.6 (43.08)^{efg}
11	Biorakshak 2%	25.72(30.47)^{e}	32.5 (34.75)^{d}	37.81(37.92)^{g}
12	Control	5.31(36.64)^{f}	5.62(7.87)^{f}	6.56 (8.49)^{h}
	CD(0.05)	4.782	5.428	6.288

Figures in parentheses are angular transformed values
Mean of three replications

Table 10. Effect of chitin and chitosan on mortality of *Helicotylenchus* sp. under laboratory conditions

Sl.no	Treatments	Cumulative mortality (%) at 24 h interval		
		Day 1	Day 2	Day 3
1	Chitin 3%	2.0^{cd}	3.33^{c}	4.0^{de}
2	Chitin 5%	2.0^{cd}	3.33^{c}	4.6^{cd}
3	Chitin 7%	3.3^{abc}	7.33^{b}	8.0^{bc}
4	Chitosan 3%	2.66^{bc}	6.0^{b}	8.0^{bc}
5	Chitosan 5%	4.6^{ab}	6.0^{b}	8.0^{bc}
6	Chitosan 7%	5.3^{a}	6.6^{b}	8.6^{cd}
7	Chitosan gel 3%	3.3^{abc}	4.0^{c}	6.0^{bcd}
8	Chitosan gel 5%	5.3^{a}	9.3^{a}	14.6^{a}
9	Chitosan gel 7%	4.0^{abc}	9.3^{a}	12.6^{a}
10	Bioboost 2%	2.6^{bc}	3.3^{c}	4.0^{de}
11	Biorakshak 2%	2.0^{cd}	3.3^{c}	4.0^{de}
12	Untreated check	0^{d}	0^{d}	0.6^{e}
	CD(0.05)	2.446	1.861	3.599

Mean of three replications

A day after treatment, chitin 7% recorded highest mortality of 66.87 per cent nematodes which was superior to all other treatments. This was followed by chitosan 7% which recorded 57.91 per cent mortality. Chitosan 5% ranked third with 50.41 per cent mortality. The corresponding mortality in chitin 3% and chitin 5% was 48.5 and 48 per cent respectively, which were statistically on par. The effect of Chitosan 3% and Bioboost 2% were not dissimilar. The mortality recorded was 38.75 and 35.62 per cent respectively, which were on parity. Biorakshak 2% caused 25.72 per cent mortality to nematodes, which was inferior to all the above treatments, but superior to chitosan gel 7% (19.46 per cent). The corresponding mortality recorded in chitosan gel 3% and 5% was 13.54 and 18.12 per cent respectively, which were on par with that of control (5.31 per cent).

Second day after treatment, chitin 7% was found to be superior to other treatments with highest mortality of 71.66 per cent. Chitin 5% and chitosan 7% resulted in 64.68 and 62.18 per cent mortality, respectively which were statistically similar. Chitosan 3% recorded 46.17 per cent mortality, while Bioboost 2% resulted in 41.25 per cent death. The corresponding mortality in Biorakshak 2% was 32.5 per cent. Chitosan gel 3, 5 and 7% caused least mortality of 15.52, 21.56 and 20.83 per cent respectively, which were on par. The least mortality (5.62 per cent) was noted in control.

On the third day, all the treatments were significantly different from each other and chitin 7% was found to be the best, resulting in 78.54 per cent mortality to the nematodes. Chitin 5%, chitosan 7%, chitin 3% and chitosan 5% and chitosan 3% were ranked sequentially recording 74.16, 66.97, 63.85, 62.91 and 55.72 per cent respectively. All the three concentrations of chitosan gel and the commercial formulations, Bioboost 2% and Biorakshak 2% did cause a mortality of 50 per cent or less, the range being 7.8 and 50.62. In the untreated group the mortality recorded was 6.56 per cent.

4.1.2.3 Helicotylenchus sp.

None of the treatments were effectual in causing mortality of *Helicotylenchus* as indicated in Table 10. The mortality rate ranged from 2 to 5.3 on first day, 3.33 to 7.33 on the second day and 4.0 to 14.6 on the third day, with no mortality in control till the end of third day, where a negligible level of 0.6 was noted. Variation exhibited by different treatments was inconsistent all through the three days.

4.1.3 Safety to Natural Enemies

Grubs and adults of the coccinellids, *Chilomenes sexmaculata* F. and *Coccinella transversalis* F, maggots of the syrphids, *Ischiodon scutellare* F. and *Xanthogramma scutellare* Thorell as well as the spiders *Tetragnatha mandibulata* Walckenaer and *Tetragnatha maxillosa* Thorell, did not exhibit any behavioural, abnormalities or symptoms or death, when treated with various formulations of chitin and chitosan.

4.2 EFFICACY OF SELCTED FORMULATIONS OF CHITIN AND CHITOSAN IN POT CULTURE

The promising treatments selected from laboratory studies were evaluated in pot culture experiments to assess their impact on leaf area damage or population build up of the test organism, incidence of other pests and diseases as well as on the growth and yield of crop plants.

4.2.1 *D. indica*

4.2.1.1 Feeding Inhibition

The effect of three superior treatments (chitin 7%, chitosan 7% and chitosan gel 7%) selected from laboratory experiment was evaluated in pot culture by raising bitter gourd.

The reduction in leaf area damage observed on third, fifth and seventh day after treatment is presented in Table 11. The results revealed that there is no

Table 11. Effect of chitin and chitosan on leaf area damage of *D. indica* under pot culture

Sl. no	Treatments	Reduction in leaf area damage (%) over control					
		First spray			Second spray		
		3DAT	5DAT	7DAT	3DAT	5DAT	7DAT
1	Chitin 7%	0.16	0.214	0.311	0.155	0.254	0.186
2	Chitosan 7%	0.31	0.368	0.32	0.225	0.341	0.240
3	Chitosan gel 7%	0.30	0.186	0.234	0.145	0.173	0.177

Mean of four replications

Table 12. Effect of chitin and chitosan on other pests and diseases in bitter gourd under pot culture

Sl. no	Treatments	Damage intensity (score) *E. septima*	Disease index (viral mosaic)
1	Chitin 7%	2	36.66 (37.01)[a]
2	Chitosan 7%	2	22.66 (28.37)[b]
3	Chitosan gel 7%	1	38.66 (38.44)[a]
4	Untreated check	2	42.33 (40.56)[a]
5	CD(0.05)		6.761

Mean of four replications

Figures in parentheses are angular transformed values

significant reduction in the feeding activity. Maximum inhibition noted was only less than one per cent.

4.2.1.2 Incidence of Other Pests and Diseases

Incidence of the leaf beetle, *Epilachna septima* Dieke in terms of damage is expressed as scores in Table 12. Plants treated with chitosan gel 7%, showed minimum damage by *E. septima* (score 1), compared to those in chitin 7%, chitosan 7% and untreated plants (score 2).

The disease index of viral mosaic was significantly less in plants treated with chitosan 7% (26.66 per cent). The disease index did not vary significantly among chitin 7% (36.66 per cent), chitosan gel 7 % (38.66 per cent) and untreated plants (42.33 per cent).

4.2.1.2 Growth Parameters

The results furnished in Table 13 reveal there was significant variation in growth parameters among the various treatments.

4.2.1.2.1 Plant height

Two weeks after treatment, (15 DAT), chitin 7% treated plants showed maximum plant height (297.58 cm) which is superior over all the treatments, followed by chitosan 7% (277.26 cm), which ranked second. Minimum height was noted in plants treated with chitosan gel 7% (247.42 cm). Plant height in untreated check was 217.40 cm.

Four weeks after treatment (WAT), there was no significant variation in plant height, among the various treatments, height ranging from 278.22 cm to 297.58. In control, the average plant height was 230.26 cm.

Six weeks after treatment, there was a significant increase in plant height in plants treated with chitin 7% (364 cm) and chitosan 7% (337.33 cm), which were statistically similar. Plants treated with chitosan gel 7% recorded

Table 13. Effect of chitin and chitosan on growth and yield of bitter gourd infested with *D. indica* under pot culture

Sl. no	Treatments	Plant height (cm)			Number of leaves			Number of branches			Yield per plant harvest^{-1}(g)
		2WAT	4WAT	6WAT	2WAT	4WAT	6WAT	2WAT	4WAT	6WAT	
1	Chitin 7%	297.58^{a}	321.98^{a}	364.00^{a}	135.33^{a}	159.33^{a}	184^{a}	1.66	2.66	4.33	326.66^{a}
2	Chitosan 7%	277.26^{ab}	304.45^{a}	337.33^{a}	98.33^{b}	151.66^{a}	146.66^{a}	2.0	2.33	3.33	346.66^{a}
3	Chitosan gel 7%	247.42^{bc}	278.22^{a}	310.66^{ab}	119.66^{ab}	142.33^{ab}	160^{a}	1.66	2.66	3.33	290.00^{a}
4	Untreated check	217.40^{c}	230.26^{b}	258.66^{b}	94.66^{b}	106.33^{b}	131^{b}	1.33	2.33	2.66	213.33^{b}
	CD(0.05)	46.771	47.678	59.300	27.271	37.772	38.267	NS	NS	NS	71.076

Mean of four replications WAT - Weeks after treatment

310.66 cm, which is inferior to above treatments and superior to untreated check (258.66 cm).

4.2.1.2.2 Number of leaves

Significant variation in number of leaves was observed after two weeks. Maximum number of leaves (135. 33) was recorded in plants sprayed with chitin 7% followed by chitosan gel 7% (119.66). The increase noted in chitosan 7% (98.33) was on par with untreated check (94.66).

After one month there was considerable increased in number of leaves in plants treated with chitin 7% (159.33) and chitosan 7% (151.66) which were statistically on par. The least effective treatment was chitosan gel 7% (142.33). Lowest number of leaves was recorded in untreated check (106.33).

All the treated plants showed significant increase in leaves over untreated check, after six weeks. Maximum number of leaves was observed in chitin 7%, followed by chitosan gel 7% and chitosan 7% (184, 160 and 146.6 respectively). In untreated check 106.33 leaves were observed.

4.2.1.2.3 Number of branches

There was no significant difference between the treatments with respect to the number of branches at two, four and six weeks after treatment (WAT).

4.2.1.3 Yield

All the treatments were significantly superior over untreated check. Maximum yield per plant per harvest was recorded in plants sprayed with chitosan 7% (346.66 g plant^{-1} harvest^{-1}), chitin 7% (326.66 g plant^{-1} harvest^{-1}) and chitosan gel 7% (290 g plant^{-1} harvest^{-1}), which were statistically on par. Yield per plant recorded in untreated check was (213.33 g plant^{-1} harvest^{-1}).

4.2.2 H. vigintioctopunctata

To check the effect of treatments on population, two sprays were given at fortnightly interval (Table 14).

4.2.2.1 Population

4.2.2.1.1 First spraying

Significant difference in population was noted among various treatments.

The pre treatment count as well as the count taken after three days of treatment did not vary. At 5 DAT, population was minimum in plants treated with chitosan gel 7% and chitosan 7%, which were statistically similar, with a mean population of 3.10 and 5.15 per plant respectively, while in control it was 9.33 per plant. Seven days after treatment, chitosan gel 7% was the most effective treatment with 2.86 insects plant^{-1}, followed by chitosan 7% that recorded 4.83 insects. The population in untreated check was 9.09.

4.2.2.1.2 Second spraying

Population did not vary among themselves, in pre treatment as well on third day after treatment. After 5 days, population recorded in chitosan 7% (0.88) and chitosan gel 7% (1.44) were on par and significantly superior to that in control (2.99). After seven days, lowest population was recorded in chitosan gel 7% (0.83), which is on par with chitosan 7% (0.83), while in control it was 2.77 per plant.

4.2.2.2 Incidence of Other Pests and Diseases

Table 15 denotes the level of damage intensity of leaf weevil, *M. viridanus*. The damage was less in plants treated with chitosan 7% (score 1), compared to those in chitosan gel 7% and untreated check (score 2).

Table 14. Effect of chitosan on population of *H. vigintioctopunctata* under pot culture

Sl. no	Treatments	Mean population (grubs and adults) plant^{-1}							
		First spray				Second spray			
		Pre count	Post count			Pre count	Post count		
			3DAT	5DAT	7DAT		3DAT	5DAT	7DAT
1	Chitosan 7%	5.75 (2.19)	5.48 (1.93)	5.15 $(1.87)^{b}$	4.83 $(1.98)^{ab}$	3.77 (1.93)	2.44 (1.50)	1.44 $(1.17)^{c}$	1.33 $(1.12)^{b}$
2	Chitosan gel 7%	5.64 (2.32)	3.21 (1.74)	3.10 $(1.71)^{b}$	2.86 $(1.66)^{b}$	3.10 (1.76)	2.10 (1.38)	0.88 $(0.93)^{b}$	0.83 $(0.91)^{b}$
3	Untreated check	6.43 (2.40)	9.43 (3.00)	9.33 $(2.99)^{a}$	9.09 $(2.69)^{a}$	3.44 (1.84)	2.66 (1.62)	2.99 $(172)^{a}$	2.77 $(1.66)^{a}$
	CD (0.05)	NS	NS	1.065	0.791	NS	NS	0.230	0.267

Figure in parentheses are square root transformed values NS – Non significant Mean of five replications

Table 15. Effect of chitosan on incidence of other pests of brinjal in pot culture

Sl.no	Treatments	Damage intensity (score)		
		Myllocerus viridanus	*Aphis gossypii*	*Leucinodes orbanalis*
1	Chitosan 7%	1	1	1
2	Chitosan gel 7%	2	1	1
3	Untreated check	2	2	1

Mean of five replications

Table 16 Effect of chitosan on growth and yield of brinjal infested with *H. vigintioctopunctata* under pot culture

Sl. no	Treatments	Plant height (cm)			Number of leaves			Number of branches			Yield (g) plant $^{-1}$ harvest^{-1}
		2WAT	4WAT	6WAT	2WAT	4WAT	6WAT	2WAT	4WAT	6WAT	
1	Chitosan 7%	50.77^{a}	54.84^{a}	59.88^{b}	47.62^{a}	56.15^{b}	59.19^{b}	5.20^{a}	5.33^{a}	10.06^{a}	149.69^{a}
2	Chitosan gel@7%	53.25^{a}	56.07^{a}	61.83^{a}	40.0^{b}	61.31^{a}	63.05^{a}	4.83^{a}	6.00^{a}	9.50^{a}	130.32a^{b}
3	Untreated check	40.71^{b}	41.53^{b}	53.33^{c}	30.08^{c}	39.83^{c}	43.39^{c}	3.75^{b}	4.33^{b}	7.56^{b}	107.87^{b}
	CD (0.05)	6.141	5.739	0.933	5.282	2.510	2.126	0.866	0.838	0.574	28.508

Mean of five replications WAT - Weeks after treatment

Damage by *Aphis gossypii* Glover was comparatively less in plants treated with chitosan 7% and chitosan gel 7% (score 1) to untreated check (score 2).

Damage of shoot and fruit borer, *Lecinodes orbanalis* Guenee, showed no difference between the treatments.

4.2.2.3 Growth Parameters

Table 16 reveals the efficacy of chitin and chitosan on growth parameters of brinjal.

4.2.2.3.1 Plant height

The plants sprayed with chitosan gel 7% and chitosan 7% were superior over untreated check, the average height being 50.77, 53.25 and 40.71 cm respectively, two weeks after treatment (WAT). Same trend was continued at four and six WAT, the average height being 56.07 cm, 54.84 cm and 41.53 cm respectively. At six WAT, treatment with chitosan gel 7% recorded maximum height of 61.83 cm, followed by chitosan 7% (59.88 cm). The average height of 56.97 cm was recorded in the control plants.

4.2.2.3.2 Number of leaves

Number of leaves was maximum in plants treated with chitosan 7% (47.62), followed by chitosan gel 7% (40). Minimum number of leaves was recorded in untreated check (30.08), at two WAT. By four weeks, chitosan gel 7% recoded maximum number of leaves (61.31), followed by chitosan 7% (56.15). Significantly less number of leaves was recorded in untreated check (39.83). After six weeks too, the same trend was noticed with 63.05, 59.19 and 43.39 number of leaves in chitosan gel 7%, chitosan 7% and untreated plants, respectively.

4.2.2.3.3 Number of branches

The number of branches was observed to be statistically similar to each other in both the treatments at two, four and six weeks after treatment, the range

being 5.2 to 10.06 in chitosan 7% and 4.83 to 9.5 in chitosan gel 7% during the time span of two weeks to six weeks. Lowest number of branches recorded in untreated check ranged from 3.75 to 7.56.

4.2.2.3.4 Yield

At the end of three pickings, chitosan 7% recorded highest yield (149.69 g) plant^{-1} harvest^{-1}, followed by chitosan gel 7% which recorded 130.32 g. Significantly less yield was observed in untreated check (107.84 g).

4.2.3 A. craccivora

Efficacy of chitin 7%, chitosan 7%, chitosan gel 7% and Biorakshak 2%, tested against *A. craccivora* on cowpea is presented in Table 17.

4.2.3.1 Population

4.2.3.1.1 First spraying

Population of aphids did not vary significantly before treatment.

Three days after treatment, the population varied significantly among treatments. Least population was noticed in plants treated with chitin 7% (66.3) which was on par with that of chitosan gel 7% (71.83). This was followed by the population in plants treated with chitosan 7% and Biorakshak 2% with 104.56 and 111.88 aphids plant^{-1} respectively, which were statistically similar. Highest population was observed in untreated check (197.98).

After five days also minimum population was noticed in plants treated with chitin 7% (39 aphids plant^{-1}) being superior to other treatments. The population in plants treated with chitosan gel 7% was 71.83, followed by chitosan 7% which recorded 80.85 aphids plant^{-1}. Treatment with Biorakshak 2% was less effective (98.96) but superior to the population in untreated check (181.36). Similar trend was noticed on the seventh day. Minimum population was recorded in chitin 7% (32.97), followed by that in chitosan gel 7 % (64.47) which

Table 17. Effect of chitin and chitosan on population of *A. craccivora* in cowpea under pot culture

Sl.no	Treatments	Mean population plant^{-1}							
		First spray				First spray			
		Pre count	Post count			Pre count	Post count		
			3DAT	5DAT	7DAT		3DAT	5DAT	7DAT
1	Chitin 7%	129.11 (2.06)	66.3 (1.82)^{c}	39 (1.59)^{c}	32.97 (1.44)^{c}	80.36 (1.64)	49.76 (1.48)	32.81 (1.34)	25.46 (1.26)^{b}
2	Chitosan 7%	164.95 (2.2)	104.56 (2.0)^{a}	80.85 (1.89)^{b}	73.53 (1.83)^{b}	50.95 (1.68)	49.81 (1.68)	40.75 (1.59)	36.26 (1.55)^{b}
3	Chitosan gel 7%	172.35 (2.18)	71.83 (1.82)^{c}	64.43 (1.72)^{bc}	64.47 (1.57)^{bc}	65.81 (1.36)	44.2 (1.27)	41.83 (1.26)	32.81 (1.20)^{b}
4	Biorakshak 2%	214.4 (2.301)	111.88 (2.04)^{a}	98.96 (1.98)^{ab}	80.93 (1.89)^{b}	83.9 (1.76	54.4 (1.54)	52.03 (1.53)	34 (1.42)^{b}
5	Untreated check	164.95 (2.20)	197.98 (2.29)^{a}	181.36 (2.24)^{a}	197.98 (2.29)^{a}	89.43 (1.89)	183.56 (2.22)	183.73 (2.2)	183.6 (2.22)^{a}
	CD (0.05)	NS	0.168	0.278	0.379	NS	NS	NS	0.626

Figures in parentheses are logarithmic transformed values Mean of three replications

NS - Non significant DAT - Days after treatment

was superior to chitosan 7% (73.53) and Biorakshak 2% (80.93). Maximum population was noted with untreated check (197.78).

4.2.3.1.2 Second spraying

Treatments were non significant before the application of treatments as well as on the third and fifth days. On the seventh day lowest population was noted in plants treated with chitin 7% (25.46) which is on par with that of chitosan gel 7% (32.81), Biorakshak 2% (34.00) and chitosan 7% (36.26). Highest population was recorded in untreated check (183.6).

4.2.3.2 Incidence of Other Pests and Diseases

Table 18 denotes the damage caused by *Tetranychus urticae*. Chitin 7%, chitosan gel 7% and Biorakshak 2% treated plants showed less damage (score 1) compared to chitosan 7% and untreated check (score 2).

4.2.3.3 Growth Parameters

Effect of treatments on growth and yield of cowpea is presented in Table 19.

4.2.2.3.1 Plant height

There was no difference in the height of the plants, among different treatments till four WAT. After six weeks of treatment all the treatments were superior to untreated check. Chitin 7%, Biorakshak 2%, chitosan gel 7% and chitosan 7% recorded 34.12, 33.16, 33.36 and 32.16 cm respectively, which were statistically similar. Plant height in untreated check was 28.75 cm.

4.2.2.3.2 Number of leaves

No significant difference was observed till four WAT. After six weeks number of leaves was maximum in chitin 7% treated plants (17.35), followed by that in Biorakshak 2% treated plants (16.5). Plants treated with chitosan gel 7%

Table 18. Effect of chitin and chitosan on other pests of cowpea under pot culture

Sl.no	Treatments	Damage intensity (score)
		Tetranychus urticae
1	Chitin 7%	1
2	Chitosan 7%	2
3	Chitosan gel 7%	1
4	Biorakshak 2%	1
5	Untreated check	2

Mean of three replications

Table 19. Effect of chitin and chitosan on growth and yield of cowpea infested with *A. craccivora* under pot culture

Sl .no	Treatments	Plant height (cm)			Number of leaves			Number of branches			Yield (g) plant^{-1} harvest^{-1}
		2WAT	4WAT	6WAT	2WAT	4WAT	6WAT	2WAT	4WAT	6WAT	
1	Chitin 7%	29.66	31.33	34.12^{a}	14.83	15.16	17.35^{a}	2.83	3.66	4.33^{a}	58.00^{a}
2	Chit osan 7%	27.33	31.83	32.16^{a}	13.00	14.50	14.33^{bc}	2.50	2.88	3.83^{ab}	45.11^{ab}
3	Chitosan gel 7%	31.70	33.36	32.83^{a}	14.50	15.66	16.00^{abc}	2.66	3.0	3.16^{b}	39.67^{b}
4	Biorakshak 2%	27.83	30.83	33.16^{a}	14.66	15.33	16.50^{ab}	2.50	3.0	3.50^{b}	39.77^{b}
5	Untreated check	23.50	26.50	28.75^{b}	12.50	13.16	13.66^{c}	2.16	2.50	3.16^{b}	35.77^{b}
	CD (0.05)	NS	NS	3.409	NS	NS	2.569	NS	NS	0.746	8.818

Mean of three replications NS - Non significant WAT - Weeks after treatment

was inferior (16) to the above treatments and superior to chitosan 7% (14.33). Minimum number of leaves was observed in untreated check (13.66).

4.2.2.3.3 Number of branches

There was no significant difference till four WAT. On six WAT superior treatment was chitin 7%, which recorded an average of 4.33 branches. Chitosan 7% ranked next (3.83). Chitosan gel 7%, Biorakshak 2%, and untreated plants were on par with 3.5, 3.16 and 3.16 number of branches, respectively.

4.2.2.3.4 Yield

After final harvest, highest yield plant^{-1} harvest^{-1} was recorded in chitin7% (58 g) which is superior over all the treatments, followed by that in chitosan 7% (45.11 g). Yield recorded in Biorakshak 2% (39.77 g), chitosan gel 7% (39.67 g) and control (35.57 g).

4.2.4 *M. incognita*

4.2.4.1 Population

Table 20 reveals the effect of different treatments on population of *M. incognita* from soil as well as root samples

4.2.4.1.1 Soil

Population of nematodes from 200 g soil sample taken from the root zone of the plants treated with chitin 7% (7g kg $^{-1}$), chitosan 7% (7g kg $^{-1}$) and chitosan gel 7% (7g kg $^{-1}$), revealed that chitin 7% and chitosan 7% were equally effective, the number of nematodes being 36 and 52.13, respectively. Among the treatments chitosan gel 7% was less effective (162.06) but superior to untreated check (321.33).

Table 20. Effect of chitin and chitosan on population of *M. incognita* under pot culture

Sl.no	Treatments	Number of nematodes	
		Soil sample	Root sample
1	Chitin 7g kg $^{-1}$	36.0 (5.95)^{c}	278.66 (16.62)^{c}
2	Chitosan 7g kg $^{-1}$	52.13 (7.21)^{c}	563 (23.72)^{b}
3	Chitosan gel 7g kg $^{-1}$	162.06 (16.17)^{b}	554.33 (23.53)^{b}
4	Untreated check	321.33 (23.30)^{a}	1589.66 (39.70)^{a}
	CD(0.05)	3.199	3.125

Mean of four replications

Table 21. Effect of chitin and chitosan on incidence of other pest and diseases of tomato under pot culture

Sl.no	Treatments	Damage intensity (score)	Disease index
		L. trifolii	Cercospora leaf spot
1	Chitin 7g kg $^{-1}$	2	33.90^{c}
2	Chitosan 7g kg $^{-1}$	3	40.89^{b}
3	Chitosan gel 7g kg $^{-1}$	3	34.55^{bc}
4	Untreated check	4	48.50^{a}
	CD(0.05)		6.714

Mean of four replications

94

4.2.4.1.2 Root

Population of nematodes in 5 g of root sample was least in chitin 7% (278.66) and was statistically superior over all the treatments. The effect of chitosan 7% (563) and chitosan gel 7% (554.33) was on par. The number of nematodes noted in untreated plants was 1589.

4.2.4.2 Incidence of Other Pests and Diseases

The damage intensity of American serpentine leaf miner, *Liriomyza trifolii* Burgess was least (Table 21), in plants treated with chitin 7% (scale 2), followed by chitosan 7% and chitosan gel 7% (score 3 each). Leaf miner damage was maximum in untreated plants (score 4).

The disease index of cercospora leaf spot was least in chitin 7% (33.90 per cent) followed by chitosan gel 7% (34.55 per cent). Chitosan 7% treated plants noted 40.89 per cent, which was inferior to above treatments and superior to untreated check (48.50 per cent).

4.2.4.3 Growth Parameters

Analysis of data revealed that there was no significant difference in plant height or number of leaves among various treatments. No significant difference between the treatments (Table 22).

4.2.4.3.1 Number of branches

Maximum number of branches (7.33) was noted with chitosan 7% treated plants, which is on par with chitin 7% (7.16). This followed by chitosan gel 7% which recorded 5.16 branches. Least number of branches was observed in untreated check (5.0).

4.2.4.3.4 Yield

All the treatments were superior over untreated check. Plants treated with chitin 7%, chitosan gel 7% and chitosan 7% recorded 459 g, 441 g and

Table 22. Effect of chitin and chitosan on growth and yield of tomato infected with *M. incognita* under pot culture

Sl. no	Treatments	Plant height (cm)	Number of leaves	Number of branches	Yield (g) plant^{-1}
1	Chitin 7g kg $^{-1}$	61.000	32.835	7.000^{a}	459.165^{a}
2	Chitosan 7g kg $^{-1}$	59.335	31.165	7.335^{a}	422.000^{a}
3	Chitosan gel 7g kg $^{-1}$	59.245	37.665	5.165^{b}	441.832^{a}
4	Untreated check	51.335	28.335	5.000^{b}	240.000^{b}
	CD(0.05)	NS	NS	1.289	144.309

Mean of four replications NS - Non significant

422 g plant^{-1} respectively, which were statistically superior. Lowest yield plant^{-1} was noted untreated check (240 g plant^{-1}).

4.3 FIELD EFFICACY OF CHITIN AND CHITOSAN IN PEST MANAGAEMENT

The most promising treatments from pot culture studies were evaluated in the most vulnerable test organisms *viz., A. craccivora* and *M. incognita*, under field conditions.

4.3.1 *A. craccivora*

Results of the experiment are presented in Table 23.

4.3.1.1 Population

The population did not vary significantly before treatment.

On the third day after treatment, the mean population did not vary significantly among the plants treated with chitin 7%, chitosan 7% and chitosan gel 7%, the population being 313.5, 263.95, and 313.5 respectively (per sampling unit), which was on par with that of control (313.5). There was complete control of aphids in the plots treated with Dimethoate 30 EC@ 0.2% (chemical check).

On the fifth day, plots treated with chitin 7%, chitosan 7% and chitosan gel 7% (189.62, 214.4 and 214.41 respectively) did not vary significantly among themselves, but was superior to the control in which the mean population was 313.5 aphids. The population recorded in dimethoate 30 EC @ 0.2% treated plants was zero.

By the seventh day there was significant reduction in population in plots treated with chitin 7% (164.82), followed by chitosan 7% and chitosan gel 7% (189.62 and 214.4 respectively). The population recorded in control was highest (289.17) and that in dimethoate 30 EC @ 0.2% it was nil.

Table 23. Effect of chitin and chitosan on population of *A. craccivora* under field conditions

Sl, no	Treatments	Pre Count	3DAT	5DAT	7DAT	10DAT
1	Chitin 7%	313.5 (17.70)	214.4 (14.45)^{a}	189.62 (13.47)^{a}	164.85 (12.71)^{b}	140.07 (11.73)^{b}
2	Chitosan 7%	313.5 (17.70)	288.72 (16.95)^{a}	214.41 (14.24)^{a}	189.62 (13.47)^{ab}	164.9 (12.71)^{b}
3	Chitosan gel 7%	263.95 (15.59)	214.4 (14.24)^{a}	214.4 (14.24)^{a}	214.4 (14.44)^{ab}	189.7 (13.68)^{b}
4	Dimethoate 30EC @ 0.2%	263.95 (15.59)	0 (0.70)^{b}	0 (0.701)^{b}	0 (0.7)^{c}	3.4 (1.20)^{c}
5	Untreated check	313.5 (17.70)	313.5 (17.72)^{a}	313.5 (17.72)^{a}	289.17 (16.95)^{a}	264 (16.19)^{a}
	CD(0.05)	NS	3.580	4.955	3.719	2.397

Figures in parentheses are square root transformed values NS - Non significant
Mean of four replications Plot size - 1m x 1m

Table 24. Effect of chitin and chitosan on yield of cowpea under field conditions

Sl.no	Treatments	Yield (kg plot^{-1})	Yield (t ha^{-1})
1	Chitin 7%	1.76^{a}	17.6
2	Chitosan 7%	1.46^{b}	14.6
3	Chitosan gel 7%	1.11^{b}	11.11
4	Dimethoate 30EC @0.2%	1.80^{a}	18.0
5	Untreated check	1.02^{c}	10.2
	CD(0.05)	0.144	

Mean of four replications Plot size - 1m x 1m

98

At the end of the observation period (10^{th}day), all the treatments were on par and significantly superior to control (264.00) the population being 140.0, 164.9 and 189.7 respectively for chitin 7%, chitosan 7% and chitosan gel 7%. Least population (3.4) was observed in plots treated with dimethoate 30 EC @ 0.2%.

4.3.1.2 Yield

Data on yield per plot presented in Table 24 reveals that, among the treatments, chitin 7% recorded highest yield (1.76 kg $plot^{-1}$), followed by those recorded in plots treated with chitosan 7% (1.46 kg $plot^{-1}$) and chitosan gel 7% (1.11 kg $plot^{-1}$). The yield recorded in chemical treatment was the highest (1.80 kg $plot^{-1}$) and that recorded in untreated, the lowest (1.02 kg $plot^{-1}$).

4.3.2 *M. incognita*

The results revealing the efficacy of chitin and chitosan in the management of nematodes is presented in Table 25.

4.3.2.1 Population

4.3.2.1.1 Soil

There was no significant variation in population before treatment. Among the treatments, plots treated with chitin 7g kg^{-1}, recorded minimum population of 152.5 nematodes, which was superior to chitosan 7g kg^{-1} (318.25). The population recorded in cartap hydrochloride 4G @ 1 kg a.i.ha^{-1} treated plots was lowest (72.25) and it was highest in the untreated plots (532.75).

4.3.2.1.2 Root

Population of female nematodes in 5 g of root sample was lowest in plots treated with chitin 7g kg^{-1} (38.75), followed by that in chitosan 7g kg^{-1} (85.5). The plots treated with cartap hydrochloride 4G @ 1 kg a.i.ha^{-1}, recorded least population of 5.5. Highest population was noted with untreated check (123.75).

Table 25. Effect of chitin and chitosan on population of *M. incognita* in tomato under field conditions

Sl. no	Treatments	Number of nematodes in 200 g of soil sample		Number of nematodes in 5 g of root sample
		Initial population	Final population	
1	Chitin 7g kg $^{-1}$	667.83 (25.57)	152.5 $(12.30)^{c}$	38.75 $(6.22)^{c}$
2	Chitosan 7g kg $^{-1}$	700.66 (26.46)	318.25 $(17.83)^{b}$	85.5 $(9.24)^{b}$
3	Cartap hydrochloride @1kg a.i ha $^{-1}$	730.83 (27.02)	72.25 $(8.32)^{d}$	5.5 $(2.32)^{d}$
4	Untreated check	678.08 (25.98)	532.75 $(22.97)^{a}$	123.75 $(11.12)^{a}$
	CD(0.05)	NS	2.581	0.361

Figures in parentheses are square root transformed values Plot size - 2m x 2m
NS - Non significant Mean of five replications

Table 26. Effect of chitin and chitosan on incidence of other pests and diseases in tomato under field conditions

Sl. no	Treatments	Damage intensity (score)	Damage index		
		L. trifolii	Bud necrosis virus*	Leaf curl virus*	Cercospora leaf spot**
1	Chitin 7g kg $^{-1}$	2	11.11 (3.40)	22.22 $(4.64)^{ab}$	39.40 (38.63)
2	Chitosan 7g kg $^{-1}$	3	11.11 (3.13)	13.88 $(3.68)^{c}$	38.34 (38.25)
3	Cartap hydrochloride 4G @ 1kg a.i ha^{-1}	2	5.5 (2.32)	16.66 $(3.97)^{bc}$	38.21 (38.16)
4	Untreated check	3	13.88 (3.41)	27.77 $(5.15)^{a}$	49.96 (44.97)
	CD(0.05)		NS	0.774	NS

Mean of five replications Plot size - 2m x 2m NS - Non significant
* Figures in parentheses are square root transformed values
**Figures in parentheses are arc sin transformed values

100

4.3.2.2 Incidence of Other Pests and Diseases

Intensity of damage caused by other pests and diseases were recorded as scores (Table 26).

The damage intensity of serpentine leaf miner, *L. trifolii* was least in plants treated with chitin 7g kg^{-1}(score 2) which was on par with cartap hydrochloride 4G @ 1 kg a.i.ha^{-1} (score 2). The damage noticed in plants treated with of chitosan 7% was similar to that observed in the untreated plots (score 3, each).

No significant difference was observed in Bud necrosis incidence and cercospora leaf spot. The disease incidence of leaf curl virus was significantly less in plants treated with chitosan 7g kg^{-1} (13.88 per cent) followed by cartap hydrochloride 4G @ 1kg a.i ha^{-1} (16.66 per cent). The damage incidence of chitin 7g kg^{-1} was 22.22 per cent, which was inferior to above treatments and superior to untreated check (27.77 per cent).

4.3.2.3 Yield

Data on yield per plot is furnished in Table 27. Among the treated plots, highest yield was recorded in plots treated with chitin 7g kg^{-1} (2.39 kg $plot^{-1}$) which was superior to all other treatments. The yield recorded in chitosan 7g kg^{-1} (1.41 kg $plot^{-1}$) cartap hydrochloride 4G @ 1 kg a.i.ha^{-1} and untreated check (1.44 and 1.10 kg $plot^{-1}$), was statistically similar.

4.4 COST ANALYSIS

4.4.1 Cowpea

The data on cost analysis for insect pest management in cowpea using chitin and chitosan is represented in Table 28.

Among the treatments, chitin 7% recorded maximum B: C ratio (3.35), followed by chitosan 7% (2.89). Chitosan gel 7% recorded minimum B: C ratio of 2.09. Highest B: C ratio of 4.51 was noted in dimethoate 30 EC @ 0.2%.

Table 27. Effect of chitin and chitosan on yield of tomato

Sl.no	Treatments	Yield (kg plot^{-1})	Yield (t ha^{-1})
1	Chitin 7g kg $^{-1}$	2.39^{a}	5.97
2	Chitosan 7g kg $^{-1}$	1.14^{b}	2.85
3	Cartap hydrochloride@ 1kg a.i ha^{-1}	1.44^{b}	3.6
4	Untreated check	1.10^{b}	2.75
	CD (0.05)	0.402	

Mean of five replications Plot size - 2m x 2m

Table 28. Cost analysis for insect pest management in cowpea using chitin based formulations

Sl. no	Treatments	Total cost excluding treatment cost (Rs ha^{-1})	Treatment cost (Rs ha^{-1})	Total cost of cultivation (Rs ha^{-1})	Grass income (Rs ha^{-1})	B:C ratio
1	Chitin 7%	157208	52806	210014	704000	3.35
2	Chitosan 7%	157208	44306	201514	584000	2.89
3	Chitosan gel 7%	157208	55066	212274	444400	2.09
4	Dimethoate 30EC@ 0.2%	157208	2266	159474	720000	4.51

Table 29. Cost analysis for nematode management in tomato using chitin based formulations

Sl. no	Treatments	Total cost excluding treatment cost (Rs ha^{-1})	Treatment cost (Rs ha^{-1})	Total cost of cultivation (Rs ha^{-1})	Grass income (Rs ha^{-1})	B:C ratio
1	Chitin 7%	147450	199090.5	346540.5	238000	0.68
2	Chitosan 7%	147450	516590.5	664040.5	114000	0.171
3	Cartap hydrochloride 4G@ 1 kg a.i ha^{-1}	147450	2653	150103	144000	0.95

4.4.2 Tomato

The variation in B: C ratio of chitin and chitosan in nematode management is presented in Table 29. All the treatments including nematicide, recorded a B: C ratio < 1. B: C ratio calculated for chitin 7% was 0.68 and that of chitosan 7% was 0.171. Chemical treatment with cartap hydrochloride 4G @ 1 kg a.i ha^{-1} recorded highest B: C ratio of 0.95.

Discussion

5. DISCUSSION

Globally increasing concern on environment safety, necessitates the need to develop biocompatible products for eco friendly pest management. The possibility of utilizing biopolymers in pest management is seen less exploited till date. Chitin is the second most abundant natural biopolymer next to cellulose. Main source of chitin is exoskeletons of crabs, insects, prawns, cell wall of fungi and gut wall nematodes (Gohel *et al.*, 2006). One of most relevant and deacetylated form of chitin is chitosan. These natural biopolymers which are abundant in nature remain unutilized or rather less utilized in the field of agriculture. Therefore, their potential in the management of insect and nematode pests as well as diseases need to be evaluated in order to evolve novel pesticides which are biodegradable and environment friendly. The present investigation was thus aimed to study the pesticidal attributes of chitin and chitosan at varying concentrations. Apart from the insecticidal, nematicidal and antimicrobial properties, its safety to non target organisms and plant growth promoting capability were also assessed.

Preliminary screening carried out under laboratory conditions revealed that, out of the six different insect pests and three different plant parasitic nematodes evaluated, the insect pests *viz.*, pumpkin caterpillar *Diaphania indica* Saunders (Crambidae : Lepidoptera), the leaf beetle *Henosepilachna vigintioctopunctata* F. (Coccinellidae : Coleoptera) and the pea aphid *Aphis craccivora* Koch (Aphididae : Hemiptera), and two of the nematodes *viz.*, the root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood, *Rotylenchulus reniformis* Linford and Oliveira were vulnerable to various chitin and chitosan formulations. However, these were less effective to the cut worm *Spodoptera litura* F. (Noctuidae : Lepidoptera), the leaf weevil *Myllocerus viridanus* Schoenherr (Curculionidae : Coleoptera), the pod bug *Riptortus pedestris* F. (Alydidae : Hemiptera) and the spiral nematode

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Helicotylenchus sp. The difference in vulnerability of various pests within the same order is evident in this study. Similar observations were reported by Zhang *et al.* (2003), wherein three insects *viz.*, American boll worm *Helicoverpa armigera* Hubner, beet army worm *Spodoptera exigua* Hubner and diamondback moth *Plutella xyllostella* L. exhibited various level of vulnerability to chitosan and oligo chitosan. *P. xyllostella* was more susceptible when compared to the others.

Among the various insects tested, significant level of feeding inhibition was noted only in *D. indica.* The feeding inhibition noted in *S. litura* (0.70 per cent), *H. vigintioctopunctata* (5.37 per cent) and *M. viridanus* (3.32 per cent) was negligible. Chitosan 7% was the best feeding inhibitor, followed by chitosan gel 7% and chitin 7%. Lower concentration (5%) of these formulations was less inhibitory and the lowest concentration (3%) was least inhibitory. The commercial formulation based on chitosan, *viz.*, Bioboost 2% and that based on chitin, *viz.*, Biorakshak 2% were also less inhibitory. Moreover, it was observed that the feeding inhibition was maximum (6.71 to 43.42 per cent) on the first day after treatment (DAT), which gradually decreased to 0.9 to 14.2 per cent on the third day.

Zeng *et al.* (2012) reported that increase in concentration of chitosan from one to five per cent increased the antifeedant rates of artificial diet fed to the black cut worm *Agrotis ipsilon* Hufnagel, pod borer *Maruca vitrata* F. and soyabean aphid *Aphis gossypii.* The maximum antifeedant effect was noticed in *M. vitrata* (87.24 per cent) followed by *A. ipsilon* (82.89 per cent) and *A. gossypii* (80.21 per cent). They suggested that chitosan as signal molecule plays a barrier function in feeding behaviour, which causes the unusual discharge of the nervous system and prevent animals from getting correct information of taste.

Contrary to the present finding that *S. litura* is not vulnerable to none of the chitin and chitosan formulations, Badawy *et al.* (2012), observed inhibitory action of the artificial diet mixed with 0.4 per cent (4g kg^{-1}) of low molecular

weight (2.27 x 10^5g mol^{-1}) chitosan (Ch1) which resulted in 76 per cent feeding inhibition on the seventh DAT and that the metal complexes of Ch 1 - Ni and Ch1 - Hg was much higher, 90.2 and 86.8 per cent respectively. The high molecular weight chitosan (9.47 x 10^5g mol^{-1}) was less effective with 35.4 per cent feeding inhibition.

The non inhibitory nature of the chitosan used in the present study may be due to the variation in the chemical forms. Different derivatives have different level of insecticidal effects. Apart from the diversified chemical forms, the molecular weight of chitosan also determines its antifeedant activity. The chitosan used in the present study might have been of high molecular weight.

On assessing the insecticidal activity of chitin and chitosan at varying concentrations (3, 5, and 7%) and as chitosan gel (3, 5 and 7%) as well as the commercial formulations, Biorakshak 2% and Bioboost 2%, it was observed that, *H. vigintioctopunctata* and *A. craccivora* were vulnerable to the formulations exhibiting varying levels of mortality. Chitosan gel 7% was the best treatment causing 100 per cent mortality on seventh day in *H.vigintioctopunctata* grubs. Bioboost 2% was the next effective treatment causing 93.33 per cent mortality. Chitosan 5% and chitosan 7% were equally effective causing 86.66 per cent mortality each. Except chitin 3% and Biorakshak 2% (26.66 and 33.33 per cent mortality respectively) all other treatments were effective resulting in more than 40 per cent mortality on the seventh day.

In a similar work conducted by Zhang *et al.* (2003), insecticidal activity of chitosan (3g L^{-1}) was noted in *H. armigera* and *P. xyllostella* with 40 and 72 per cent mortality respectively after three days. Different aphid species *viz.*, *Rhopalosiphum padi* L. *Sitobion avenae* F. *Metopolophium dirhodum* Walker, *M. persicae*, *Hyalopterus prun* Goffroy and *A. gossypii*, treated with chitosan 600 to 6000 mg L^{-1} also exhibited insecticidal activity with 60 - 80 per cent mortality after three days. They suggested that the insecticidal activity might be due to the

formation of a film which acts as a barrier on its surface which blocks the entry of air into spiracles, leading to death. Another hypothesis is that, chitosan might have induced the activity of chitosanase in the insect body which causes death of the treated insects.

In the case of *A. craccivora*, chitin 7% was the best treatment causing 100 per cent mortality on fifth day. Biorakshak 2% was the next effective treatment causing 91.66 per cent mortality. Except the above treatments, all others took five days to cause 80 per cent mortality.

None of the earlier researchers have reported the insecticidal activity of chitin, whereas there are few reports which indicated the insecticidal activity of chitosan as well as its complexes with different metal complexes.

It was first demonstrated by Zhang *et al.* (2003), that the flowers sprayed with chitosan at 600 to 6000 mg L^{-1} resulted in 93 to 99 per cent mortality in mealy plum aphid *H. pruni* and 70 - 80 per cent mortality in corn leaf aphid *R. padi*, rose grain aphid *M. dirhodum* and cotton aphid, *A. gossypii*. Rabea *et al.* (2006), observed that synthetic diets mixed with five per cent of chemically modified derivatives of chitosan, *viz.*, N - (3 - phenylbutyl) chitosan, *N* - tridecanylchitosan and *N* - (phenylethyl) chitosan resulted in 50, 47 and 37 per cent mortality respectively in the third instar larvae of *S. litura*. However, they pointed out that, larvae treated with *N* - propylchitosan, *N* - undecanylchitosan and *N* - (3 - phenylpropyl) chitosan, exhibited two to three times reduction in growth and that they affected normal ecdysis.

Similar observations were recorded in a bioassay carried out by Badawy and El -Aswad (2012), they observed that 2.27×10^5 g mol^{-1}, 3.60×10^5g mol^{-1} and 5.97×10^5g mol^{-1} molecular weight of chitosan (1000 mg L^{-1}) caused 96, 87, and 100f per cent of mortality respectively in oleander aphid *Aphis nerii* Boyer de Fonscolombe. Among the various chitosan - metal complexes tested,

chitosan - Cu complex resulted maximum mortality of 70, 73 and 94 per cent of mortality respectively at 250, 500 and 1000 mg L^{-1}. The other complexes with Ag, Ni and Hg caused mortality varying from 42.4 to 83.1 per cent, the highest being that observed for chitosan - Ni complex. They also demonstrated the insecticidal activity of chitosan in *S. litura*, where it was found that the artificial diet containing 0.4 per cent (4g kg^{-1}) of low molecular weight (2.27 x 10^5g mol^{-1}) chitosan (Ch1) caused 50 per cent mortality on seventh DAT and that the mortality recorded in metal complexes of Ch 1 - Ni and Ch1 - Hg was much higher, *i.e.* 93.3 and 83.3 per cent mortality respectively.

In the present investigation it was observed that, though chitin and chitosan had insecticidal activity in some insects *viz.*, *H. vigintioctopuncata* and *A. craccivora*, in the other test insects *D. indica*, *S. litura*, *M. viridanus* and *R. pedestris* there was no mortality at all. This dissimilarity observed within the same insect order needs thorough investigation on the factors that affect their antifeedant and insecticidal properties.

Nanoparticles of chitosan mixed with synthetic diet was found to be promising in reducing the fecundity of cowpea weevil, *Callosobruchus maculates* F. (10.9 per cent reduction) and in pulse beetle, *Callosobruchus chinensis* L. (21.9 per cent reduction). Niether mortality nor antifeedant activity was reported in these insects. Such detailed studies which could disclose the changes in biology or metabolism of the pests would certainly be beneficial in revealing the actual mode of action of these biopolymers.

In the *in vitro* experiment on plant parasitic nematodes to evaluate the effect of various chitin and chitosan formulations *viz.*, chitin (3, 5 and 7%), chitosan (3, 5 and 7%) and chitosan gel (3, 5 and 7%) as well as commercial formulations, Bioboost 2% and Biorakshak 2% it was disclosed that the *M. incognita* was highly susceptible to chitin and chitosan, while *R. reniformis* was moderately susceptible and *Helicotylenchulus* sp. was least affected.

In general, chitosan 5% and 7 % were the best treatments (Fig. 1) that could result in considerable level of inhibition to *M. incognita* eggs (66.67 per cent on fourth day). Chitin 7% and chitosan 3% also exhibited significant level of inhibition (53.34 and 40 per cent each). The effect of lower concentrations of chitin, chitosan gel, Bioboost and Biorakshak were comparatively low (13.33 to 33.33 per cent). J_2 was the most vulnerable stage, wherein a higher death rate of 95.75 to 100 per cent was noted on the third day, with all the formulations tested.

Khalil and Badawy (2012), also reported the nematicidal effect on *M. incognita* juveniles under *in vitro* conditions. They found that low molecular weight chitosan (2.27×10^5 g mol^{-1}) recorded high nematicidal activity (LC_{50} - 124.90 mg L^{-1}) than high molecular weight chitosan (9.47×10^5 g mol^{-1}) with an LC_{50} value of 260.08 mg L^{-1}, at 48 hours after treatment. While testing different concentrations of chitosan against *M. javanica*, Sayed *et al.* (2014), found that low molecular weight chitosan (50 kDa) as well as high molecular weight (470 kDa) chitosan completely inhibited the egg hatching, under *in vitro* conditions.

In the case of *R. reniformis,* chitin 7% was found to be superior rather than chitosan 7% and chitosan gel 7%. Chitin 7% recorded 78.4 per cent mortality while chitosan 7% recorded 66.97 per cent and chitosan gel 7%, 50.62 per cent mortality, on the third day.

Perusal of literature does not reveal any research reports on the efficacy of these formulations on *R. reniformis* and *Helicotylenchulus* sp.

Pot culture experiments carried out with formulations selected from laboratory experiment, in bitter gourd, brinjal, cowpea and tomato revealed the efficacy of treatments in managing the population of the test organisms, their

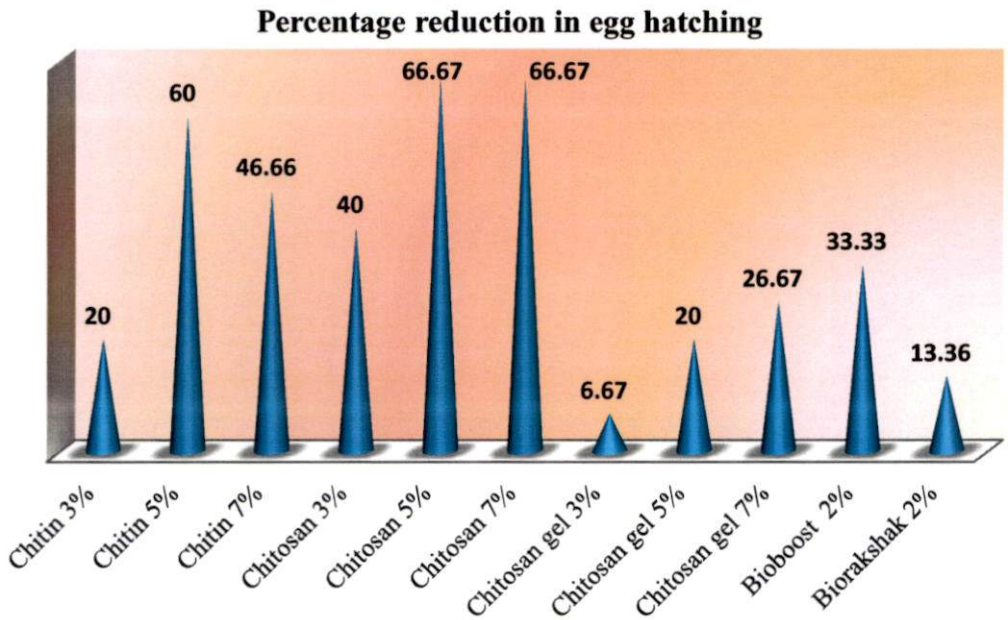

Fig. 1 Effect of chitin and chitosan formulations on hatching of *M. incognita*

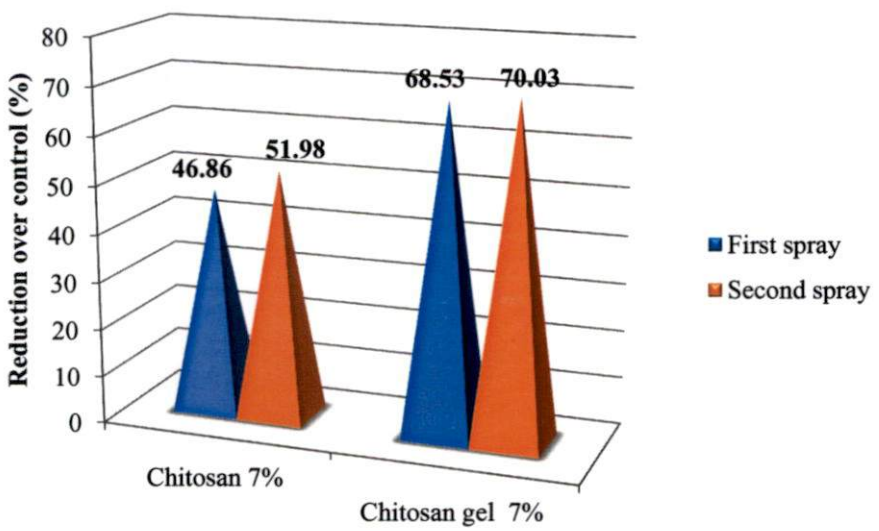

Fig. 2 Effect of chitosan formulations on population of *H.vigintioctopunctata* (7DAT), under pot culture

efficacy in reducing the damage caused by other pests and diseases as well as their impact on growth and yield parameters of the crops.

In bitter gourd, there was no feeding inhibition in *D. indica* with none of the treatments (chitin 7%, chitosan 7% and chitosan gel 7%), while under laboratory conditions there was 43.43 per cent inhibition. This difference may be due to the difference in method of treatment. Under *in vitro* conditions, the leaf dip method might have resulted in more deposits of chitin /chitosan on it, while the spray method might have resulted in less deposit of the treated particles. The incidence of other pest, *Epilachna septima* Dieke was reduced in plants treated with chitosan gel 7% while the incidence noted in chitin 7% and chitosan 7%, treatments was on par with that of control.

In brinjal, chitosan gel 7% and chitosan 7 % were equally effective in managing the population of *H. vigintioctopunctata* (Fig. 2). Maximum reduction in population over control was 51.98 and 46.86 per cent with chitosan gel 7% and chitosan 7% on the seven DAT. These results were in agreement with the observations of the laboratory experiment. Regarding the incidence of other pests *viz.*, *M. viridanus* and *A. gossypii,* though chitosan gel 7% was not effective for *M. viridanus*, it reduced the incidence of *A. gossypii.*

In cowpea, population of pea aphid *A. craccivora* (Fig. 3) was much reduced in plants treated with chitin 7%, percentage reduction over control being 83.34 per cent on the seven DAT, while that in plants treated with chitosan gel 7% was 67.43 per cent. Apart from aphids, the incidence of spider mite *Tetranychus* sp. was also found to be reduced with all the formulations tested.

In tomato, percentage reduction in population of *M. incognita* (Fig. 4) was least in chitin 7% treated plants (88.87 and 82.47 per cent respectively in soil and root samples), followed by chitosan 7% (83.77 and 64.58 per cent respectively). Incidence of other pests *viz., L. trifolii* was least in plants treated with chitin 7%

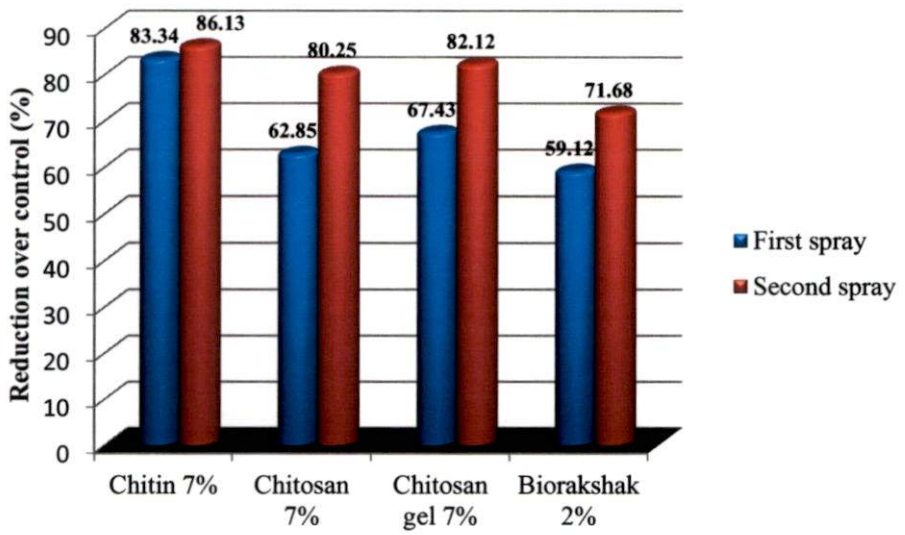

Fig. 3 Effect of chitin and chitosan on population of *A. craccivora* (7DAT), under pot culture

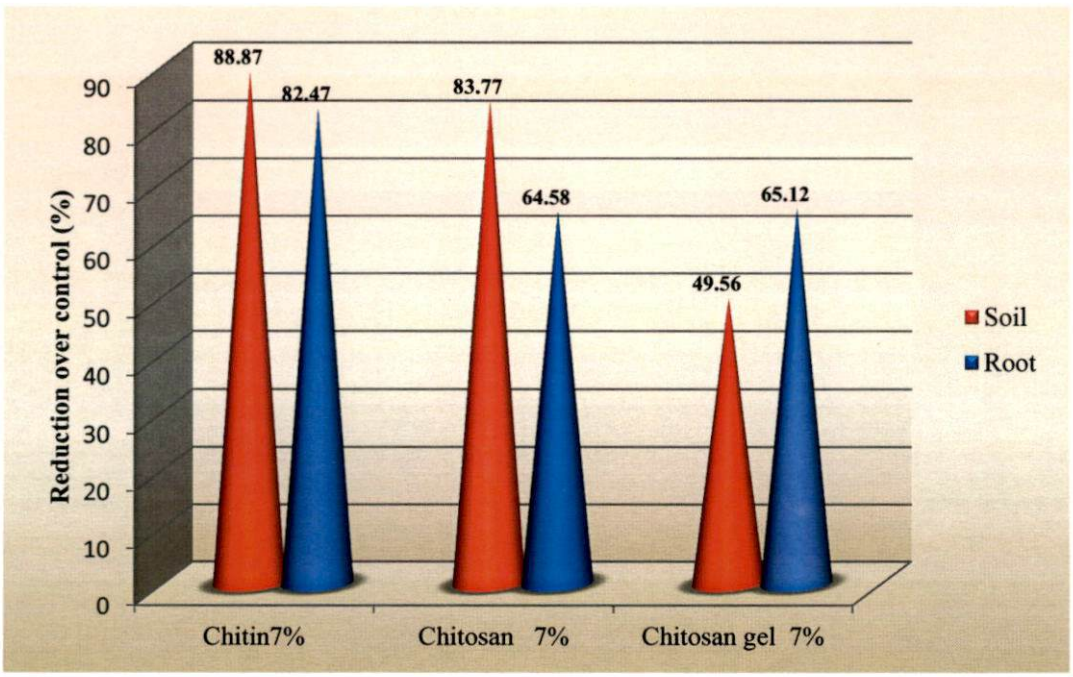

Fig. 4 Effect of chitin and chitosan on population of *M. incognita,* under pot culture

and less in those treated with chitosan 7% and chitosan gel 7%, compared to untreated plants.

Perusal of literature revealed that research works are scanty on pot culture studies that assessed the efficacy of chitin and chitosan formulations on the incidence of insect pests. Even so, Senthilraja *et al.* (2010) reported that, in pot culture experiment, foliar application of talc based formulations of *Beauveria bassiana* (Balsamo) Vuillemin and *Pseudomonas fluorescens* (Flugge) Migula amended with chitin (1%) recorded lowest incidence (2.5%) of ground nut leaf miner, *Aproaerema modicella* Dev. compared to that in chemical check (5%) and control (31.5 per cent). They accounted the suppressive action, to the probable systemic resistance induced by chitin against insect pests.

The efficacy of chitin and chitosan formulations in reducing the population of *M. incognita*, noticed in the present investigation is supported by the findings of various researchers. Mian *et al.* (1982) reported that, potting mixture amended with 1 - 4 % chitin (w/w) in green house cultivation did not produce any *Meloidogyne arenaria* galls in squash plants, when observed after six weeks. They also found that, increase in concentration of chitin, increased the activity of aryl phosphatise, chitinase and urease in soil. The nematicidal action of chitin was ascribed to the high levels of ammonia released into the soil through decomposition of the biopolymer in soil. Ammonical nitrogen at levels higher than 500 kg ha^{-1} is toxic to certain nematode species. Furthermore, it was demonstrated that soil amendment with chitin increased soil mycoflora that are capable of degrading chitin. This chitinolytic activity was noticed in *Fusarium solani* (Mart.) Sacc, *Fusarium udum* Butler, *Humicola fuscoatra* Traaen and *Pseudeurotium ovale* Stock which were found to be parasitic on eggs of *M. arenaria* and *Heterodera glycines* Ichinohe.

Similar results were also reported by Culbreath *et al.* (1985). They found that soil amended with chitin 2% with or without hemicellulose suppressed *M. arenaria* juveniles as there were no or galls or juveniles in the soil samples,

while in untreated plants the juvenile population was 42 per 100 cm^3 of soil. An increase in number and activity of specialized mycoflora rather than an increase in general fungal activity is likely responsible for extended control of plant parasitic nematodes observed in soil amended with chitin. They also attributed the release of ammonia as one of the reasons for mortality.

Spiegel *et al.* (1986 ; 1987) reported that potting mixture amended with ClandoSan prepared from crustacean chitin at the rate of 0.05 to 0.3 per cent reduced the gall index of *M. javanica* in bean and tomato plants either by releasing ammonia or by increasing the chitinolytic microorganisms in soil. Likewise, Mittal *et al.* (1995) found that, potting mixture consisting of chitin (0.5 g) and the nematophagus fungus, *Paecilomyces lilacinus* (Thom) Samson considerably reduced the *M. incognita* galls g^{-1} in brinjal (9.84 g root fresh $weight^{-1}$), tomato (3.51 g root fresh $weight^{-1}$) and bengal gram (1.42 g root fresh $weight^{-1}$) than in control plants (330.67, 80.82 and 70.92 respectively g root fresh $weight^{-1}$) after 90 days of inoculation.

Not only chitin, but chitosan was also found to be effective in reducing *M. incognita* infestation in tomato as observed in this study. Parallel observations were reported by Radwan *et al.* (2012), wherein the potting mixture amended with different concentrations (1, 3, 5 and 10 g kg^{-1}) of chitin and chitosan appreciably reduced the *M. incognita* root galls (58.79 and 72.03 per cent respectively) and juvenile population (51.43 and 69.87 per cent respectively). Comparable results were also reported by Escuderoa *et al.* (2016) who found that application of chitosan (2 mg L^{-1}) significantly increased the egg parasitisation of *M. javanica* by nematophageous and endophytic fungus *Pochonia chlamydosporia* (Goddard) Zare and Gams.

Experiments carried out in field for confirmation of results, once again revealed the superiority of chitin 7% (46.94 per cent reduction in population) over chitosan 7% (37.53 per cent) and chitosan gel 7% (28.14 per cent) in managing

A. craccivora (Fig. 5). Efficacy of chitosan in managing aphid population was earlier reported by Cardenas *et al.* (2002) in wheat and Cabrera (2003), in sugar beet. Iriti and Faoro (2009) explained the defence mechanism in plants treated with chitosan. The defence responses elicited by chitosan in plants include, raising of cytosolic Ca^{2+}, activation of MAP - kinases, callose apposition, oxidative burst, hypersensitive response and synthesis of abscissic acid, jasmonate, phytoalexins and pathogenesis related proteins. These findings were further supported by Zeng *et al.* (2012) who reported that soybean seeds treated with 5% chitosan reduced the emergence of *A. nerii* by 35.16 per cent by three weeks and by 84.46 per cent by six weeks. This insecticidal activity of chitosan was attributed to the stimulation of systemic antibodies in treated plants. Faoro (2013) reported fifty per cent reduction in population of *M. persicae*, in bean field drenched with chitosan at the rate of 0.1 per cent, five DAT.

In the field experiment too, significant reduction of *M. incognita* (Fig. 6) population was noted in plots treated with chitin 7% after three months. The percentage reduction was 71.66 in soil samples and 68.68 per cent in root samples respectively, while in chitosan 7% treated plots the corresponding reduction was 54.50 and 30.90 per cent respectively. *L. trifolii* damage was also less in chitin 7% treated plots, which was comparable to plots treated with cartap hydrochloride 4G @ 1 kg a.i. ha^{-1}.

Nematicidal activity of chitin on *M. incognita* under field conditions was first reported by Mankau and Das, (1969; 1974) and later by Westerdhal *et al.* (1992) and Hallmann *et al.* (1999) and Ladner, (2008).

Apart from *M. incognita*, chitosan was reported to be effective in suppressing the population of other plant parasitic nematodes like *H. trifolii*, *Pratylenchus* sp. as well as *Paratrichodorus minor* (Colbran) Siddiqi in white clover and ryegrass (Bell *et al.*, 2000).

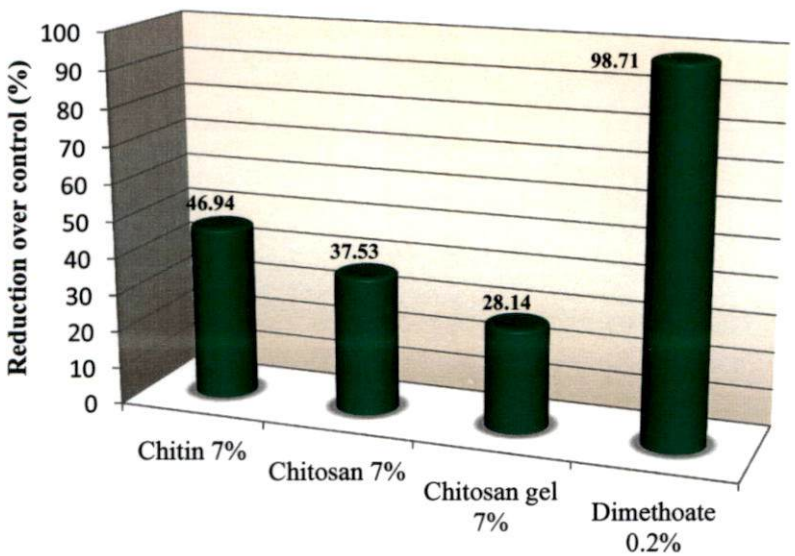

Fig. 5 Effect of chitin and chitosan on population of *A. craccivora*

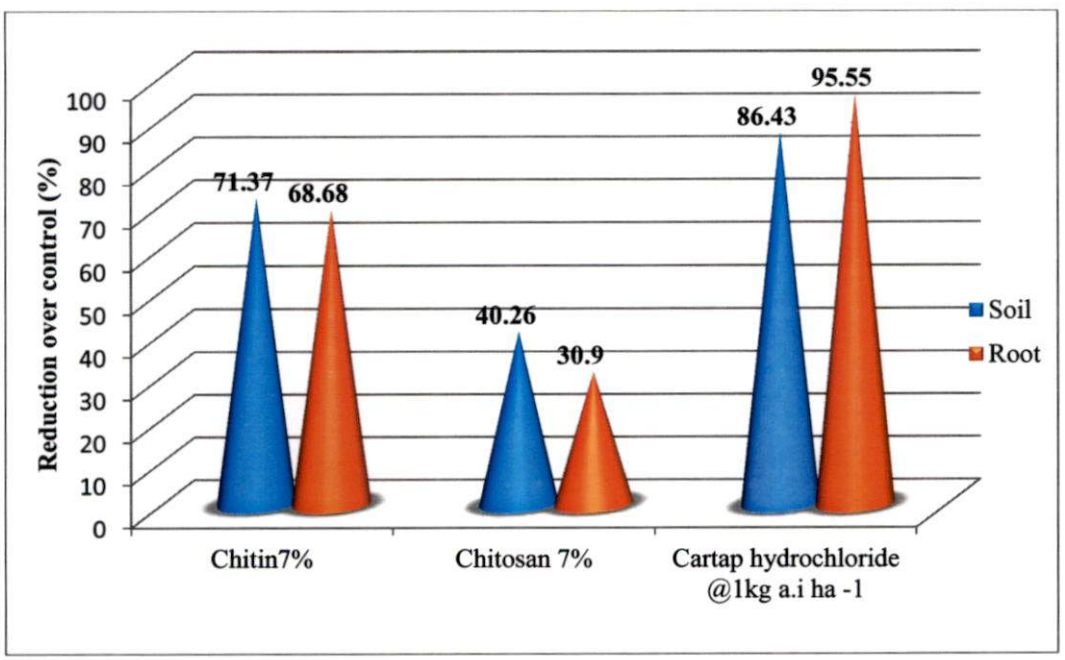

Fig. 6 Effect of chitin and chitosan on population of *M. incognita*

Evaluation of chitin and chitosan formulations for their potential in plant disease control, proved the efficacy of chitosan in reducing the damage caused by yellow mosaic virus in bitter gourd, leaf curl virus and cercospora leaf spot in tomato.

Antifungal property of chitin and chitosan was studied by researchers worldwide on various crop plants. Bell *et al.* (1998) reported that, celery seedlings dipped in chitosan (30 %) and planted in chitin (1 kg per experimental plot - 6m x 1m) amended soil reduced fusarium wilt by 52.3 per cent when compared to control (90.5 per cent), two months after planting. Its ability to control Botrytis bunch rot in grapes was reported by Aziz *et al.* (2006) wherein, they reported 65 per cent control of the disease in plants treated with chitosan @ 150 mg L^{-1}. It was also found to increase the lipoxygenase, phenylalanine ammonia lyase (PAL) and chitinase activity which imparts immunity to plants. El - Moughy (2006), found that plants treated with a combination of chitin (6 g kg^{-1}) and chitosan (6 g kg^{-1}) significantly reduced the root rot pathogens *viz.*, *Rhizoctonia solani* Kuhn, *Fusarium solani* (Mart.) Sacc. and *Sclerotium solani* Sacc. Palma-Guerrero *et al.* (2010), pointed out that sensitivity of phytopathogenic fungus to chitosan might be due to the increase in its plasma membrane fluidity. Sathiyabama *et al.* (2014) reported 75 per cent suppression of *Alternaria solani* (Ellis and Martin) Sorauer symptoms in tomato plants treated with chitosan (1mg mL^{-1}) five days later. They also noted an increase in chitinase activity in leaves.

The antiviral property of chitin and chitosan was previously reported by Zhao *et al.* (2007) who observed 75 per cent reduction in damage of tobacco mosaic virus (TMV) in plants treated with 50 µg m L^{-1} oligo chitosan, when compared to control. Furthermore, treated plants recorded a marked increase in the level of nitrous oxide, hydrogen peroxide , phenylalanine ammonia - lyase and chitinase mRNA, within 12 h of treatment which plays an important role in developing immunity to TMV. Tomato plants treated with chitosan (5%) enriched pseudomonas, significantly reduced 80.33 to 96.33 per cent of leaf curl

disease severity in tomato, under field conditions. It also increased polyphenol oxidase activity in the treated plants (Mishra *et al.*, 2014). Noiket *et al.* (2014) reported that tomato plants treated with 10, 20, 40 and 60 ppm of chitosan exihibited tomato yellow leaf curl virus symptoms 7-14 days later compared to control.

Rabea *et al.* (2003) opined that, antimicrobial activity of chitosan might be due to the stimulation in synthesis of phenolic acids, especially ferulic acid. The synthesis of precursors of lignin such as p - coumaric acid, sinapic acids and phenolic acids having antimicrobial activity was also stimulated by chitosan treatment.

Investigation on effect of chitin and chitosan on growth parameters of crop plants too, furnished encouraging results. Generally chitin 7% and chitosan 7% were effective in increasing plant growth parameters like number of leaves, number of branches, plant height and yield in brinjal, cowpea, bitter gourd and tomato (Fig. 7). There was 38.76 to 117.27 per cent increase in yield in plants treated with chitin 7% and 20.81 to 75.83 per cent increase in chitosan 7% treated plants.

Findings of this study were supported by those of Kalaiarasan *et al.* (2008). They reported significant increase in shoot length (32.33 cm) and root length (12.67 cm) when compared to untreated check in ground nut (19.33 cm and 8.70 cm), when the potting mixture was amended with 1.2 % chitin. Further, Gaun *et al.* (2009), observed that seed treatment of chitosan @ 0.25 % increased the shoot length (10.80 cm) as well as root length (13.48 cm) in maize over control (7.23 and 12.47 cm respectively). Zakaria *et al.* (2009) reported an increase in plant height, number of leaves and yield in potato when culture medium was added with 500 mg L^{-1} of chitosan. Similar results were reported in the case of blueberry (Cabrera *et al.*, 2010); okra and tomato (Mondal *et al.*, 2012) and cowpea (Farouk and Amany, 2012). In rice, Boonlertnirun and Suvannasara (2012) observed that foliar application of mixed fertilizer with chitosan

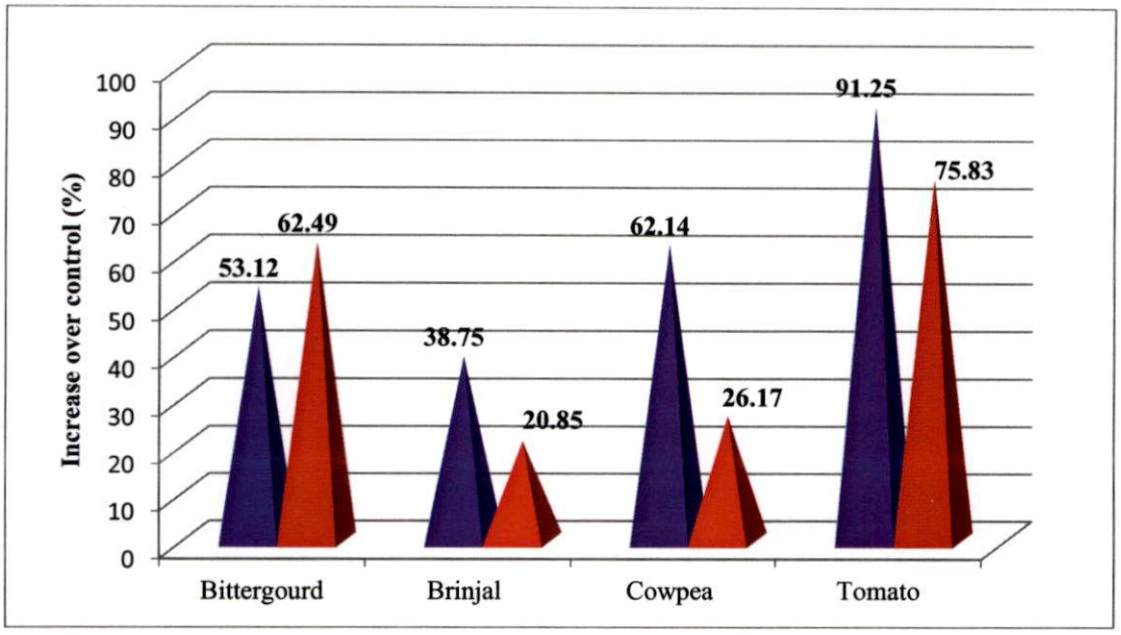

Fig. 7 Effect of chitin and chitosan on crop yield

(80mg L^{-1}) enhanced the plant height, number of tillers and panicles, and yield when compared to control.

As a part of the present investigation, safety test was carried out in the predatory coccinellids, *Chilomenes sexmaculata* F. and *Coccinella transversalis* F. syrphids *Ichiodon scutellare* F. and *Xanthogramma scutellare* Thorell as well as the spiders *Tetragnatha mandibulata* Walckenaer and *T. maxillosa* revealed that, the formulations had no negative effects on these natural enemies. The treated insects did not show any behavioural abnormalities or death.

Palma - Guerrero *et al.* (2008), demonstrated that chitosan was much safe to entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin and nematophagous fungus, *Pochonia clamydosporia* (Goddard) Zare and Gams. The Environment Protection Agency (2008) concluded that chitin and chitosan were non toxic to human health as well. Radwan *et al.* (2012), found that chitin and chitosan (1 - 10 g kg^{-1}) were not phytotoxic to tomato plants.

Chitin enriched formulations of the entomopathogenic fungi *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno, and *Lecanicillium lecanii* (Zimm.) Zare and Gams, at 10^9 spores mL^{-1} were found to be safe to the coccinellids, *Micraspis discolor* F. and *Coccinella transversalis* F., the mirid, *Cyrtorhinus lividipennis* Reuter, the carabid, *Ophionea nigrofasciata* Schmidt - Gobel, and the spiders *Tetragnatha maxillosa* Thorell and *Oxyopes shweta* Tikader (Sankar, 2017).

Cost analysis revealed that foliar application of chitin, chitosan and chitosan gel are economic for insect pest management, while for nematode management where they are required in more quantities for soil amendment, they are not economic. Processes that can bring down the cost of recovery of these biopolymers from the shrimp and crab shells, would make nematode management using these organic products, much cheaper.

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From the present study, it is apparent that the natural biopolymers, chitin, chitosan and chitosan gel are effective to *H. vigintioctopunctata*, *A. craccivora and M. incognita* at 7% concentration. Foliar application of bioformulations, chitin 7% and chitosan 7% increased the plant growth and yield. Field application of chitin 7% effectively controlled *M. incognita* population in tomato and reduced *A. craccivora* population in cowpea.

Summary

6. SUMMARY

Exploitation of natural biopolymers that are compatible with the environment is one of the main challenges in modern agriculture. Chitin is the most abundant natural biopolymer on the planet, next to cellulose. Chitosan, the most relevant derivative of chitin is also biodegradable, biocompatible and is non toxic to mammals. Utilization of these biopolymers in agriculture as biostimulants, will help to reduce the quantity of fertilizers and plant protection chemicals as well as elicit more healthier and sustainable organic agriculture. The present study entitled "Potential of the natural biopolymers, chitin and chitosan in pest management" was carried out during 2105 - 17, at the College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, with an aim to evaluate its insecticidal properties for utilization in crop pest management.

Preliminary screening of insect pests and nematodes for their vulnerability to various formulations of chitin and chitosan was carried out under laboratory conditions. The formulations tested were chitin (3%, 5%, 7%), chitosan (3%, 5%, 7%), chitosan gel (3%, 5%, 7%), chitin based commercial formulation, Biorakshak 2% and chitosan based commercial formulation, Bioboost 2%. They were tested for their antifeedant and insecticidal properties, by leaf dip method and spray methods in laboratory as well as by foliar spray and soil amendments to check their nematicidal and growth promoting properties, in pot and field studies.

The test organisms selected were those representing the major insect orders Lepidoptera (*Diaphania indica* Saunders and *Spodoptera litura* F.), Coleoptera (*Henosepilachna vigintioctopunctata* F. and *Myllocerus viridanus* Schoenherr), and Hemiptera (*Aphis craccivora* Koch and *Riptortus pedestris* F.), three plant parasitic nematodes *viz.*, *Meloidogyne incognita* (Kofoid and White) Chitwood, *Rotylenchulus reniformis* Linford and Oliveira and *Helicotylenchus* sp) and three group of natural enemies *viz.*, the coccinellids (*Chilomenes sexmaculata* F. and *Coccinella transversalis* F.) the syrphids

(*Ischiodon scutellare* F. and *Xanthogramma scutellare* Thorell.) and the spiders (*Tetragnatha mandibulata* Walckenaer and *Tetragnatha maxillosa* Thorell).

Laboratory studies revealed that the *D. indica*, *H. vigintioctopunctata*, *A. craccivora*, *M. incognita* and *R. reniformis* were vulnerable to different chitin and chitosan based formulations whereas, *S. litura*, *M. viridanus*, *R. pedestris* and *Helicotylenchus* sp. were not.

Antifeedant effect was determined by calculating feeding inhibition percentage using the following formula suggested by Arivoli and Tennyson, 2013. It was noticed that, the formulations were inhibitory only to the larvae of *D. indica*, chitosan 7% being the superior formulation with 43.25 per cent feeding inhibition on first day which decreased to 13.30 per cent on the fourth day. This effect of chitosan gel 7% (42.85 per cent) and chitin 7% (25.4 per cent) was also notable. Lower concentration (5%) of these formulations was inhibitory and the lowest concentration (3%) was least inhibitory. The commercial formulations based on chitosan, Bioboost 2% and that based on chitin, Biorakshak 2% were also less inhibitory similar to the lowest concentrations. Moreover, it was observed that the feeding inhibition was maximum (6.71 to 43.42 per cent) on the first day after treatment (DAT), which gradually decreased over the following days, reaching negligible rates of 0.9 to 13.90 per cent among various treatments. Larvae fed with treated leaves were inactive with less feeding activity, however none of the treatments caused mortality in both leaf dip as well as spray method.

Insecticidal activity was assessed by spray method, calculating the cumulative per cent mortality at 24 h interval. The formulations had varying levels of insecticidal effect on *H. vigintioctopunctata*, and *A. craccivora*. For *H. vigintioctopunctata*, chitosan gel 7% was the best treatment, which recorded 100 per cent mortality on seventh day, followed by Bioboost 2% (93.33 per cent). Chitosan 5% and chitosan 7% were equally effective caused 86.66 per cent mortality each. Except chitin 3% and Biorakshak 2% (26.66 and 33.33 per cent

mortality) all other treatments caused more than 40 per cent mortality on seventh day. Treated grubs become inactive as well as inhibit the feeding.

A. craccivora was the most vulnerable insect pest to chitin and chitosan based bioformulations. Chitin 7% was the best treatment causing 100 per cent mortality on fifth day. Chitin 5 %, chitosan 3%, Biorakshak 2% and Bioboost 2% ranked second with 91.66 per cent mortality. Mortality caused by chitin 5%, chitosan 3%, Bioboost 2% was 91.66 per cent each. More than 80 per cent mortality was recorded in chitosan 7%, chitosan gel 7%, chitosan 5%, chitosan gel 5% and chitosan 5% (83.33 to 90 per cent), while chitosan gel 3% caused 80 per cent.

Nematicidal effect was assessed by observing the detrimental effects on egg hatching and mortality to juveniles (J_2). Hatching inhibition was noticed in *M. incognita.* In *M. incognita* chitosan 5% and chitosan 7% were the best treatments that could result in considerable level of hatching inhibition in *M. incognita* eggs (66.67 per cent) on fourth day, followed by chitin 7% (46.66 per cent). The effect of chitin 5%, chitosan 3%, chitosan gel 7% and Bioboost 2% were comparatively low (33.33 to 40 per cent). Significantly low inhibition was recorded in chitin 3%, chitosan gel 5%, Biorakshak 2% and chitosan gel 3% (6.67 to 20 per cent). J_2 is the most vulnerable stage, wherein a higher death rate of hundred percent was resulted chitin 7%, chitosan 5%, 7% and chitosan gel 7% on the second day, which were statistically similar to chitosan 3% and Bioboost 2% (99.39 per cent each). Chitosan gel 5%, chitin 5% and Biorakshak 2% caused 95.75, 87.27 and 89.69 per cent mortality respectively while it was least in chitin 3% (72.12 per cent).

In *R. reniformis,* chitin 7% was most effective treatment, caused 78.54 per cent mortality. Chitin 5%, chitosan 7%, chitin 3% and chitosan 5% and chitosan 3% were moderately effective (55.72 to 74.16 per cent). All the three concentrations of chitosan gel, Bioboost 2% and Biorakshak 2% were least

effective (7.8 and 50.62 per cent). The treatments were ineffective to *Helicotylenchus* sp.

Screening of natural enemies *viz.*, the coccinellids (*C. sexmaculata* and *C. transversalis*) the syrphids (*I. scutellare* and *X. scutellare*) and the spiders (*T. mandibulata* and *T. maxillosa*) by topical application, revealed that none of the formulations were harmful to them.

Laboratory studies also revealed that, the bioformulations chitin, chitosan and chitosan gel were effective at 7% concentration and commercial based bioformulations, Bioboost and Biorakshak were moderately effective.

Pot culture experiments were conducted to evaluate the treatments based on the effectiveness in reducing the insect population, incidence of other pests and diseases as well as the growth parameters of crop plants. In bitter gourd, with *D. indica* as test organism, feeding inhibition was not noted, in contrary to the laboratory observations. This may probably due to the lesser amount of deposits of the treated particles while spraying, compared to the leaf dip method adopted in laboratory assay. Whereas the damage caused by another pest *Epilachna septima* Dieke was reduced in chitosan gel 7% treated plants. Moreover, viral mosaic disease index was less in chitosan 7%. Chitin 7% treated plants recorded maximum number of leaves (135. 33) and plant height (297.58 cm) followed by chitosan gel 7%, 119.66 leaves, while chitosan 7% recorded 277.26 cm plant height at two weeks after treatment. Chitosan 7% increased the yield by 62.49 per cent, chitin 7% by 53.12 per cent and chitosan gel 7% by 35.93 per cent.

In brinjal, with *H. vigintioctopunctata* as the test insect, chitosan gel 7% treated plants recorded lowest population followed by chitosan 7 % at seven days after treatment. The reduction in population noted was 46.86 and 68.53 per cent respectively. Chitosan 7% was effective in reducing damage caused by *M. viridanus* as well and *Aphis gossypii* Glover, as well. Chitosan 7% and chitosan gel 7% treated plants recorded 24.71 and 30.80 per cent increased in plant height, 58.31 and 32.91 per cent increased in leaves as well as 38.66 and

28.8 per cent increased in branches over control, at two weeks after treatment. However, chitosan 7% treated plants increased the yield by 38.78 per cent followed by chitosan gel 7%, 20.81 per cent.

In cowpea, where *A. craccivora* was the major test insect, chitin 7% was the superior treatment in reducing the population by 83.34 per cent on the seventh day, followed by chitosan gel 7%, 67.43 per cent. Chitosan 7% and Biorakshak 2% were least effective treatments. The damage caused by other pests *viz., Tetranychus* sp. was less in chitin 7%, chitosan 7% and Biorakshak 2% treated plants. The plants treated with chitin 7% and chitosan 7% recorded average plant height (34.12 and 32.16 cm respectively), number of leaves (17.35 and 14.33 respectively) and maximum number of branches (4.33 and 3.83 respectively) at six weeks after treatment. Chitin 7% enhanced the yield by 62.14 and 26.17 per cent respectively.

Pot culture studies on tomato with *M. incognita* as the test organism, revealed that, chitin 7% was the highly effective treatment in reducing the population in soil and root by 88.87 and 82.47 per cent, followed by chitosan 7% 83.57 and 64.58 per cent. The damage index of other pests *viz.*, *Liriomyza trifolii* Burgess and disease index of cercospora leaf spot was least in chitin 7% treated plants. Chitosan 7% and chitin 7% treated plants recorded maximum number of branches, 7.16 and 7.33 respectively. Chitin 7% treated plants enhanced the yield by 91.25 per cent and chitosan 7% by 75.83 per cent.

Field experiment on cowpea revealed that, chitin 7% took seven days to reduce the population of *A. craccivora* (164.82 plant^{-1}), followed by chitosan 7% and chitosan gel 7% (189.62 and 214.4 respectively), while in plots treated with dimethoate 30EC @ 0.2 % there was complete control. Yield plot^{-1} was 1.76 kg in chitin 7% and it was 1.8 kg in dimethoate 30EC @ 0.2 %.

In the field trial on tomato, population of nematodes in soil and root sample were less in plots treated with chitin 7% (152.5, 38.75) than in control plots (532.75, 123.75), while in chemical treatment with

cartap hydrochloride 4G @ 1 kg a.i.ha^{-1} it was 72.25 and 5.5 respectively. The incidence of *L. trifolii* was less in chitin 7% treated plants and that of leaf curl virus was less in treated plants when compared to control. Significantly high yield was noted with crude chitin 7g kg^{-1} (2.39 kg plot^{-1}) compared to control (1.10 kg plot^{-1}).

Thus, the investigation is concluded with the salient findings listed below

- The bioformulations of chitin, chitosan and chitosan gel were effective at 7% concentration compared to lesser concentrations.
- *A. craccivora* and *M. incognita* were the most vulnerable pests to these formulation compared to *H. vigintioctopunctata* and *R. reniformis.*
- Foliar spray of chitosan 7% was the effective treatment for managing *H. vigintioctopunctata,* while the commercial formulations Biorakshak 2% (chitin based) and Bioboost 2% (chitosan based) were moderately effective.
- Foliar spray of chitin 7% was effective in managing the population of *A. craccivora.*
- Soil amendment of chitin 7% was the best treatment that inhibited egg hatching and reduced the population of *M. incognita* in soil.
- Foliar application of chitosan 7% and chitosan gel 7% increased the growth and yield in brinjal, while chitin 7% and chitosan 7% were best for boosting growth in cowpea and tomato
- Chitin and chitosan were safe to coccinellid, syrphid and spider predators.

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POTENTIAL OF THE NATURAL BIO POLYMERS, CHITIN AND CHITOSAN IN PEST MANAGEMENT

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ABSTRACT

The investigation entitled "Potential of natural bio polymers, chitin and chitosan in pest management" was conducted at College of Agriculture, Vellayani during 2016-17 with an objective to evaluate the insecticidal properties of the natural biopolymers, chitin and chitosan for utilization in crop pest management. Various formulations tested were, chitin (3%, 5%, 7%), chitosan (3%, 5%, 7%), chitosan gel (3%, 5%, 7%), chitin based commercial formulation, Biorakshak 2% and chitosan based commercial formulation, Bioboost 2%. They were tested for their antifeedant, insecticidal, nematicidal and growth promoting properties by leaf dip method and spray methods in laboratory as well as by foliar spray and soil amendments in pot and field studies.

Laboratory studies revealed that the pumpkin caterpillar, *Diaphania indica* Saunders, the leaf beetle *Henosepilachna vigintioctopunctata* F., pea aphid *Aphis craccivora* Koch, the root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood. and the reniform nematode *Rotylenchulus reniformis* Linford and Oliveira were vulnerable to different chitin and chitosan based formulations whereas, the cut worm *Spodoptera litura* F. the leaf weevil, *Myllocerus viridanus* Schoenherr, the pod bug *Riptortus pedestris* F. and the spiral nematode *Helicotylenchus* sp. were not. All the formulations tested were safe to the coccinellid predators, *Chilomenes sexmaculata* F. and *Coccinella transversalis* F., the syrphid predators, *Ischiodon scutellare* F. and *Xanthogramma scutellare* Thorell. and the spiders *Tetragnatha mandibulata* Walckenaer and *Tetragnatha maxillosa* Thorell. Antifeedant effect was noticed only *D. indica*, chitosan 7% being the superior formulation with 43.25 per cent feeding inhibition on first day which decreased to 13.30 per cent on the fourth day. Insecticidal activity was noticed in *H. vigintioctopunctata*, chitosan gel 7%, being the best treatment causing 100 per cent mortality on seventh day, followed by Bioboost 2% (93.33 per cent mortality). In *A. craccivora*, chitin 7% caused 100 per cent mortality on fifth day while Biorakshak 2% recorded 91.66 per cent

mortality. Nematicidal effect was noticed in *M. incognita* and *R. reniformis.* In *M. incognita* hatching was reduced by 66.6 per cent for chitosan 5% and chitosan 7%, followed by chitin 7% (53.34 per cent). The juveniles (J_2) were highly susceptible to all the formulations at 7% causing 100 per cent mortality on second day. Chitin 7% caused 78.54 per cent mortality in *R. reniformis* on third day.

Pot culture studies revealed that, in brinjal, *H. vigintioctopunctata* population was lowered in plants treated with chitosan gel 7% and chitosan 7%, the population being 2.86 and 4.83 at seven days after treatment (DAT), while in control it was 9.09 per plant. Incidence of *M. viridanus* and *Aphis gossypii* Glover was also less in plants treated with chitosan 7% and chitosan gel 7%. The plant height, number of leaves and branches and were also more in the treatment, chitosan gel 7%. Highest yield was recorded in chitosan 7% (149.69 g plant^{-1} harvest^{-1}). In cowpea, *A. craccivora* population was significantly reduced (32.97) in plants treated with chitin 7% while in control it was 197.98 at seven DAT. Plants treated with chitin 7%, chitosan gel 7% and Biorakshak 2% showed less damage of mite, *Tetranychus* sp. Chitin 7% was the best treatment that favored the growth parameters in cowpea. In tomato, *M. incognita* population in soil was least in plants treated with chitin 7% and chitosan 7% (36 and 52.13) where as in root samples it was least (278.66) in chitin 7% treated plants. Incidence of *Liriomyza trifolii* Burgess and cercospora leaf spot was also less in these treatments.

Field experiment on cowpea revealed that, chitin 7% took seven days to reduce the population of *A. craccivora* (164.82 plant^{-1}), followed by chitosan 7% and chitosan gel 7% (189.62 and 214.4 respectively), while in plots treated with dimethoate 30EC @ 0.2% there was complete control. Yield plot^{-1} was 1.76 kg in chitin 7% and it was 1.8 kg in dimethoate 30EC @ 0.2%. In the field trial on tomato, population of nematodes in soil and root sample were less in plots treated with chitin 7% (152.5, 38.75) than in control plots (532.75, 123.75), while in chemical treatment with cartap hydrochloride 4G @ 1 kg a.i.ha^{-1} it was 72.25 and

5.5 respectively. The incidence of *L. trifolii* was less in chitin 7% treated plants and that of leaf curl virus was less in treated plants when compared to control. Significantly high yield was noted with crude chitin 7g kg^{-1} (2.39 kg $plot^{-1}$) compared to control (1.10 kg $plot^{-1}$).

The study indicated that the potential of natural biopolymers chitin and chitosan can be exploited for the holistic management of crop plants as it has capacity to regulate the population of insect and nematode pests as well as plant diseases. The growth and yield promoting attributes and safety to natural enemies makes them ideal candidates in integrated pests and disease management as well as integrated nematode management programmes.