BIOEFFICACY OF THE ACAROPATHOGEN, *Acremonium zeylanicum* (PETCH) GAMS AND EVANS AGAINST THE SPIDER MITE, *Tetranychus truncatus* EHARA (ACARI: TETRANYCHIDAE)

by

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(2015-11-010)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture
(AGRICULTURAL ENTOMOLOGY)

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE
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KERALA, INDIA
2017

DECLARATION

I, Alka Sherief hereby declare that the thesis entitled **"Bioefficacy of the acaropathogen, *Acremonium zeylanicum* (Petch) Gams and Evans against the spider mite, *Tetranychus truncatus* Ehara (Acari: Tetranychidae)"** is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Bioefficacy of the acaropathogen, *Acremonium zeylanicum* (Petch) Gams and Evans against the spider mite, *Tetranychus truncatus* Ehara (Acari: Tetranychidae)"** is a bonafide record of research work done independently by Ms. Alka Sherief under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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ACKNOWLEDGEMENT

"Delight yourself in the Lord, and He will give you the desires of your heart."

I humbly grab this opportunity to acknowledge reverentially, many people who deserve special mentions for their varied contributions in assorted ways that helped me during my M.Sc. research programme and in making of this thesis. I could never have embarked and finished the same without their kind support and encouragements.

I would like to acknowledge all the teachers I learnt from since my childhood, I would not have been here without their guidance, blessing and support.

First of all, I am extremely grateful to my guide, ***Dr. Haseena Bhaskar****, Professor, Department of Agricultural Entomology and the chairman of my advisory committee, for her supervision, advice, and invaluable guidance from the very early stages of this research and for giving me her extraordinary research experiences throughout the study. Her truly scientist intuition has made her as a constant oasis of ideas and passions in science, which exceptionally inspired and enriched my growth as a student, and a researcher that I want to be. I consider it my privilege to have accomplished this thesis under her right guidance. I am thankful to her more than she knows.*

I owe my most sincere gratitude to ***Dr. Mani Chellappan****, Professor and Head, Department of Agricultural Entomology and member of my advisory committee for his valuable suggestions, timely advice, friendly demeanour and brilliant supervision that have been very helpful for this study.*

My special words of thanks should also go to ***Dr. Madhu Subramanian****, Professor and Head, AICRP on BCCP and W, and member of my advisory committee for always being so kind, helpful and motivating. He has taught me another aspect of life, that, "goodness can never be defied and good human beings can never be denied". His constant guidance, cooperation, careful reading and constructive comments was valuable and has always kept me going ahead. I owe a lot of gratitude to him for always being there for me and I feel privileged to be associated with a person like him during my life.*

I am extremely indebted to ***Dr. S. Beena****, Professor, Department of Plant Pathology and member of my advisory committee for her enthusiasm, help, valuable suggestions and technical advice shown throughout the entire course of my research work.*

I would like to express great appreciation to my other teachers ***Mr. Sateeshan, Dr. Berin Pathrose, Dr. Deepthi K. B. and Dr. Smitha M. S.*** *for their expert teaching, kind treatment, moral support, valuable and constructive suggestions offered throughout the work.*

I cannot forget the help and support of our retired teachers of the Department of Agricultural Entomology. With great respect and devotion, I would like to express my deep sense of gratitude to ***Dr. Maicykutty P. Mathew, Dr. Susannamma Kurien, Dr. R. Ushakumari and Dr. Lyla K. R.***

I am obliged to ***Dr. S. Krishnan****, Professor and Head, Department of Agricultural Statistics for his assistance during the statistical analysis of the data.*

I wish to express my gratitude to ***Dr. B. Mallik, Dr. Chinnamade Gowda and Dr. N. Srinivasa****, UAS, Bangalore for offering training on Taxonomy of Phytophagous and phytoseiid mites and their technical support.*

I also gratefully acknowledge ***Vidya miss*** *for her constant advises, elderly support, personal helps, friendly nature and many more that I received during these days.*

My heartfelt thank goes to ***Akhila chechi****, Microbiologist, AICRP on BCCP and W, for her kindness, support and unflagging encouragement.*

Thank you so much to Subitha chechi, Dhanya chechi, Swathy, Anju and Sreedevi of AINPAA, who has always gone above and beyond the call of duty. I would like to extend hugs, warm thanks to my seniors Neena chechi, Aswin chetan, Najithatha, Hareesh ettan, Nasiyatha, Nesmitha, Nithya chechi, Neenu chechi, Manju akka, Uma chechi, Chandini chechi, Smitha miss for their valuable help and support. I express my thanks to juniors Kavya, Shahanaz, Nimisha, Anusree and Rejin chetan for their help and company during various stages of my course programme.

I dedicate my sincere thanks to the non-teaching staff of the department of Entomology and the contract labourers for their timely help and cooperation given during my field work.

I owe special thanks to Librarian, College of Horticulture, ***Dr. A.T. Francis*** *and all other staff members of Library, who guided me in several ways, which immensely helped for collection of literature for writing my thesis.*

I am thankful for the service rendered by ***Mr. Aravind*** *all along the academic period.*

I express my deep sense of gratitude to ***Kerala Agricultural University*** *for financial and technical support for persuasion of my study and research work.*

A very special gratitude goes out to my roommate late Femi Jose, for her warm, supporting and genuine friendship that is one of the best and out of this world. She was an inspiration. I would never be able to pay back the love and affection showered upon by her.

Without the company of friends, there is no colour for college life. I am extremely grateful to my dearest friends Anusree, Amrutha, Mohasina, Nishi, Nufaisa and Arunima for all the love, help and co-operation which they extended during this two years. They were always by my side during difficult situations and together we shared wonderful moments.

I would like to acknowledge my other friends for their moral support and motivation, which drives me to give my best. Poorni, Alfi, Aysha, Rajeswari, Anju, Jeena, Ebimol, Nimmi, Anila, Neethu ...the list is endless...thanks to one and all.

Finally, I would like to acknowledge the people who mean world to me, my loving mother, Mrs. Najumunneesa Sherief and my dear father, Mr. H. M. Sherief, in the first place are the persons who rooted the fundamentals of strength of my character, showing me the joy of intellectual pursuit ever since I was a child and raising me earnestly with their caring, gentle love and sacrifices along with my ever supportive and caring brother Shan. Thank you amma, bava and bhaiyya for showing faith in me and giving me liberty to choose what I desired. I consider myself the luckiest in the world to have such a supportive family, standing behind me with their love and support.

My special gratitude to all my cousins for being with me in thicks and thins of life, I find myself lucky to have people like you in my life.

I thank the Almighty for giving me the strength and patience to work through all these years so that today I can stand proud with my head held high. You given me the power to believe in my passion and pursue my dreams. I could never have done this without the faith I have in you, the Almighty!

ALKA SHERIEF

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Introduction

1. INTRODUCTION

Phytophagous mites are serious pests of over 200 cultivated crops, which include major food crops, vegetables, fruit crops and ornamentals. Among the different mites, spider mites (Prostigmata: Tetranychidae), cause severe injury to vegetables grown under both protected and open field conditions. The yield loss due to infestation by *Tetranychus* spp. in vegetables varies from 7- 48 per cent (Srinivasa and Sugeetha, 1999).

The tetranychid mites mostly colonize the leaves and suck sap, causing speckling and yellowing of leaves, premature leaf drop and death of plant. In severe cases, the mites web profusely and may form a thick layer of webbing that covers the entire plant. Apart from its polyphagous nature, short life cycle and high reproductive potential, factors such as change in climatic conditions and over-use of plant protection chemicals have helped the mites attain major pest status in vegetables in recent times.

There are about 37 species of mites known to feed on vegetable crops among which mites belong to the family Tetranychidae are more devastating. *Tetranychus urticae* Koch, known as two-spotted spider mite, is an ubiquitous and economically important agricultural pest feeding on a wide range of host plant species (Xie *et al.*, 2006). However, recent study indicated that *T. urticae* is being replaced by *Tetranychus truncatus* Ehara as the predominant species of mite infesting vegetable crops of Kerala (Bennur *et al.*, 2015).

Currently farmers use large quantities of synthetic acaricides for managing the mite problems because of their spectacular knock down effects. However, this often leads to adverse effects like ecological problems, resurgence, effects on non-target organisms, development of acaricide resistance, and health risks to farmers and consumers. Hence, there is a need for identifying safer strategies in mite management programmes.

Biological control, which helps obviate most of the above concerns is therefore gaining acceptance in management of arthropod pests including mites.

However, efforts at biocontrol of mites have till date been limited to use of predatory mites, coccinelids *etc*. Acaropathogenic microbes though reported have hardly been evaluated for biocontrol of mites.

Acremonium zeylanicum (Hypocreales: Hypocreaceae) (Petch) Gams and Evans is an entomopathogenic fungus known to infect a number of insect pests. Natural incidence of *A. zeylanicum* was reported on sugarcane woolly aphid from Karnataka (Tippannavar *et al*., 2006). Recently, *A. zeylanicum* was isolated from *T. urticae* infesting brinjal grown under protected cultivation in Thrissur district (Krishna *et al*., 2014) where it was observed to cause significant mortality to the spider mite. The high mortality observed, coupled with the adaptability makes the local isolate of *A. zeylanicum* an ideal candidate for further evaluation as a biocontrol agent of spider mites. However, no study on the same has been conducted on *T. truncatus*, which is a major pest of vegetables in Kerala especially under polyhouse condition.

It is in this context that the present study entitled "Bioefficacy of the acaropathogen, *Acremonium zeylanicum* (Petch) Gams and Evans against the spider mite, *Tetranychus truncatus* Ehara (Acari: Tetranychidae) was undertaken, to explore the possibilities in biological control programme with the following specific objectives

1. Evaluation of biocontrol potential of *Acremonium zeylanicum* against the spider mite, *Tetranychus truncatus* under laboratory and field conditions

2. Assessment of sensitivity of the acaropathogen to selected novel acaricides and botanicals

3. Evaluation of safety of *A. zeylanicum* to the predatory mite, *Neoseiulus longispinosus*

Review of Literature

2. REVIEW OF LITERATURE

The spider mites, belonging to family Tetranychidae, are serious agricultural pests worldwide. They cause direct as well as indirect damage to plants and are responsible for heavy losses in vegetable crops. Reliance on synthetic acaricides for mite management had led to several adverse effects such as development of resistance to pesticides and ill effects on natural enemies that regulate other pests. There is an increasing interest in natural pesticides which are derived from plants and microorganisms since they are perceived to be safer than the synthetic chemicals (Yanar *et al*., 2011). In the present study, a local isolate of *Acremonium zeylanicum*, from mycosed mites on brinjal in polyhouse at Thrissur district, Kerala by AINPAA (All India Network Project on Agricultural Acarology), College of Horticulture, Kerala Agricultural University, was evaluated for its potential in biological control. The literature pertaining to the studies on *Acremonium zeylanicum* in mite management is very scanty and hence related references of other entomopathogenic fungi for the management of mites are also reviewed below.

2.1 Management of spider mites

A brief review on use of agrochemicals and other safer strategies for management of spider mites on vegetable crops is furnished here under.

2.1.1 Conventional synthetic acaricides for management of spider mites

Synthetic acaricides were being used against mites on various crops since the time dicofol was discovered during Second World War, earlier to which elemental sulphur was used as an acaricide. After this, organochlorine compounds like endosulfan having acaricidal activity in addition to insecticidal activity was used against mite pests of crops. Later, formamidines (chlordimeform and amitraz) and sulfite esters (propargite), methamidophos and tetradifon were found effective against all stages of plant feeding mites. Even organophosphorus compounds like phorate, monocrotophos, ethion, dimethoate, triazophos, and phosalone are

effective acaricides too. Among carbamates carbofuran and aldicarb are acaricidal (Perry *et al.*, 1998). Sannaveerappanavar and Channabasavanna (1989) reported that dicofol was highly effective against all stages of *Tetranychus ludeni* when tested in the laboratory. In field and pot culture experiments conducted at Tamil Nadu Agricultural University to evaluate the bioefficacy of acaricides and neem oil against *T. urticae* on okra and brinjal, dicofol (0.05%) proved to be the most effective resulting in reduction in mite population to the tune of 70.56 to 91.85 per cent and 66.99 to 99.20 per cent on okra and brinjal respectively (Ramaraju, 2004). Siddiqui and Singh (2006) evaluated nine acaricides against *T. urticae* infesting aubergine in Varanasi, Uttar Pradesh, and found that dicofol 18.5 EC was the most promising against mites.

Resistance in mites to acaricides was first observed by Compton and Kearns (1937) in the two spotted spider mite against ammonium potassium selenosulfide (Selecide). The introduction of organophosphorus acaricides in 1947, first TEPP and later on parathion, resulted in the virtual elimination of mites in greenhouses. Resistance to parathion and TEPP became evident in 1949, and by 1950 resistant mites were present in a large percentage of rose houses in the eastern United States (Jeppson *et al.*, 1975). Patel (1982) had proved that the overuse of endosulfan or carbaryl lead to outbreaks of spider mite, *Tetranychus telarius* Linn. in eggplants grown in the field. *Tetranychus ludeni* infesting cotton had developed resistance to all organophosphate insecticides tested (Herron *et al.*, 1998). Resistance to dicofol was recorded on mite species of okra in parts of Gujarat, emphasizing the use of newer acaricidal molecules with novel mode of action (AINPAA, 2009). Synthetic pyrethroids at sub-lethal concentrations stimulated reproduction of mites leading to resurgence of mite pests and had eliminated the natural enemies of mites infesting crops, mainly vegetables like okra and brinjal (Srinivasa *et al.*, 2010). James and Prince (2002) observed that imidacloprid caused resurgence by causing an intrinsic increase in egg production in the exposed *T. urticae*. Acaricide resistance in plant-feeding mites is a seriously increasing phenomenon, especially in spidermite which has a remarkable intrinsic potential for rapid evolution of resistance (Leeuwen *et*

al., 2010). Therefore, there was a felt need for development of new acaricides with novel modes of action, which are effective against the target pests, compatible with their natural enemies, safer products with respect to human health and the environment.

2.1.2 Novel acaricides for management of spider mites

Forti *et al*. (1994) observed that fenpyroximate and pyridaben were effective against European red mite, *Panonychus ulmi* while it proved detrimental to the predatory phytoseiid *Amblyseius andersoni* under laboratory and field conditions.

Among the different acaricides tested, diafenthiuron and spiromesifen proved significantly superior over other treatments with more than 96 per cent motality on adult mites of *T. macfarlanei* on brinjal at Dharwad. This was followed by dicofol and propargite with 93.33 and 85.00 per cent mortality. Acaricidal activity of diafenthiuron, spiromesifen and dicofol increased with duration and caused cent per cent kill of mites (Patil, 2005).

Machini (2005) reported that the ovicidal effect of spiromesifen 240 SC on *T. evansi* was 100 per cent and it did not show acute toxicity on motile stages of the mites, but completely deterred the adult females from laying eggs. Fenpyroximate @ 25 g a.i.ha^{-1} significantly reduced infestation by the mite, *T. cinnabarinus* (3.06 mites/leaf) on brinjal followed by flufenzin @ 100 g a.i.ha^{-1}(9.48 mites/leaf) (Naik *et al*., 2006).

Patil and Nandihalli (2007) studied the ovicidal effects of various acaricides on *T. macfarlanei* infesting brinjal. Egg mortality of more than 98 per cent was recorded by diafenthiuron 50 WP and spiromesifen 240 SC. Fenazaquin 10 EC recorded 93 per cent egg mortality and emerged as the next best treatment.

Onkarappa *et al*. (2007) observed that spiromesifen 240 SC resulted in significant reduction in population of both egg and active stages of *T. urticae* on

brinjal after seven days of treatment, which was on par with the standard check dicofol 18.5 EC and was followed by fenazaquin 10 EC.

Singh and Choudary (2008) reported that application of propargite 57 EC @ 1500 ml ha^{-1} reduced the mite population in okra by 84.67 per cent. Spiromesifen treated *T. urticae* recorded significant decrease in fecundity and fertility of females and it also reduced the viability of eggs (Marcic *et al*., 2010).

Marcic *et al*. (2011) conducted field evaluation of spiromesifen against European red mite, *Panonychus ulmi* on apple and recorded 88.80, 94.60 and 92 per cent efficacy at 11, 22 and 32 days after treatment respectively. Fenazaquin 10 EC when tested against two spotted spider mite, *T. urticae* showed that the acaricide at 125-150g a.i. ha^{-1} recorded significantly lower mite population compared to dicofol at 250g a.i. ha^{-1}. Fenazaquin at 125 and 150g a.i. ha^{-1} treated plots observed higher fruit yield (Misra, 2011).

A field trial was conducted at vegetable research farm Ludhiana, to evaluate newer molecules, horticultural mineral oil (HMO) and NSKE. Fenazaquin 10 EC at 400 g a.i. ha^{-1} recorded 77.2 per cent reduction in egg population and was on par with dicofol. NSKE 5 per cent and HMO 1 per cent was on par with each other recording 53.84 per cent reduction in the egg counts (AINPAA, 2011).

A farm level experiment was conducted at Banglore to evaluate fenazaquin and HMO, in comparison with the conventional acaricide molecule, dicofol against spider mite infesting tomato. Reduction in mite population was recorded to the tune of 52 to 81 per cent, 24 to 72 per cent and 46 to 85 per cent in fenazaquin, HMO and dicofol respectively. Compared to other treatments fenazaquin offered control over the mite to an extended period of two weeks (AINPAA, 2011).

Bhaskar *et al*. (2012) reported an outbreak of red spider mite *Tetranychus* sp. in banana at Thrissur district of Kerala during 2012 which was effectively managed by the application of spiromesifen 240 SC (0.8 ml l^{-1}) and fenazaquin 10 EC (2 ml l^{-1}).

Efficacy of acaricides *viz*. propargite (0.057%), fenazaquin (0.0025%) and hexythiazox (0.005%) against *T. urticae* on strawberry revealed that maximum mortality of mite was observed in fenazaquin treated plants (0.2 mites per leaf) followed by propargite (0.6 mites per leaf). Hexythiazox treated plants recorded maximum mite population (9.4 per leaf). Propargite and fenazaquin proved highly effective and recorded 0.6 and 1.4 mites per leaf respectively after seven days of spray as against 27.4 and 26.7 mites per leaf in pre-count (AINPAA, 2013).

Fenazaquin 10 EC when tested along with spiromesfen 240 SC, conventional acaricides and wettable sulphur, recorded significantly lower population of *T. urticae* at two, four and seven days after treatment. Spiromesifen treatment showed high population of mites two days after treatment and was significantly high compared to other treatments. Later it was found to be on par with them at four and seven days after treatment (AINPAA, 2013).

An experimental trial was conducted at Himachal Pradesh, to assess the efficacy of three acaricides viz. hexythiaox (0.005%), fenazaquin (0.0025%) and propargite (0.057%) and a horticulture mineral oil (1%) against spider mites in beans. The study reported maximum kill of mites in fenazaquin and propargite sprayed plants compared to all other treatments (AINPAA, 2013). Reddy and Latha (2013) recorded highest mortality of *T. urticae* on ridged gourd with fenazaquin treatment (100% and 97.68%) followed by spiromesifen (97.68% and 92.1%) in first season and second season respectively.

Darandale *et al*. (2014) reported spiromesifen as an effective acaricide recording 2.95 mites per leaf at 72 h after the treatment application which had an initial pre-treatment population count of 34.87 mites per leaf and its effect continued till nine days after treatment. Efficacy of different acaricides was tested against acid lime mite *Schizotetranychus baltazari* Rimando and the results showed that spiromesifen 240 SC and difenthiuron 50 SC recorded zero population of mite at fifteen days after spraying (Kottalagi *et al*., 2014).

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Monica *et al.* (2014) observed that spiromesifen caused 100 per cent mortality of red spider mite at 72 h after spraying under laboratory condition and in field, it recorded mortality of more than 70 per cent at 3 days after spraying. Reddy *et al.* (2014) compared novel acaricides against two spotted spider mite *T. urticae* infesting cucumber (*Cucumis sativus*) under green house conditions and found that spiromesifen 22.9 SC caused the highest per cent mortality (100%) of the mites at 10 DAS.

Spiromesifen @100 g a.i.ha^{-1} reduced the overall mite population of *T. urticae* on brinjal within three days of application and caused 100 per cent mortality at 14 days after spraying (Kavya *et al.*, 2015).

Aswin *et al.* (2016) conducted laboratory bioassay to evaluate the relative toxicity of four new acaricide molecules *viz.*, fenazaquin 10 EC, spiromesifen 240 SC, fenpyroximate 5 SC and propargite 57 SC against rice leaf mite, *Oligonychus oryzae*. Fenazaquin was identified as the most effective molecule and caused 100 per cent mortality of gravid females at 24h and 86.60 per cent mortality of egg at 72 h. Spiromesifen recorded ovicidal and adulticidal effect of 80 and 100 per cent respectively at 72h after treatment.

Krishna and Bhaskar (2016) conducted field experiment to evaluate newer acaricides, fenazaquin 10 EC, diafenthiuron 50 WP and spiromesifen 240 SC against *T. urticae* on okra and recorded significant reduction in mite population of 84.43, 91.68 and 89.55 per cent in first season and 85.41, 87 and 88.5 per cent in second season respectively.

Baloch *et al.* (2016) studied the effect of different acaricides against mites in okra. Spiromesifen recorded the highest efficacy of 94.52 and 98.01 percent after 15 days of first and second spray respectively. Application of fenazaquin @ 125 a.i. ha^{-1} or spiromesifen @ 100g a.i. ha^{-1} or propargite @ 570 a.i. ha^{-1} or fenpyroximate @ 30g a.i. ha^{-1} has been found promising against rice leaf mite, *Oligonychus oryzae* and banana leaf mite causing more than 95 per cent reduction in mite population (AINPAA, 2016)

Spiromesifen 22.9 SC reported adulticidal effect on *T. urticae* with LC_{50} value of 12.53 ppm and ovicidal activity of 100 per cent at 10 DAS (Kumari *et al.*, 2017).

2.1.3 Botanicals for management of spider mites

Yathiraj and Jagadish (1999) studied the effect of various plant extracts on *T. urticae* under laboratory condition and found that seed kernel extract of neem (NSKE) has caused 60.25 per cent and 51.2 per cent mortality of the mite at 5 per cent and 1 per cent respectively. When different neem formulations and castor oil were evaluated for their efficacy against the red spider mite, *T. urticae* in the laboratory following dip method, neem oil was found to cause significantly higher mortality (79.60%) compared to other treatments (Umamaheshwari *et al.*, 1999).

In an experiment conducted at Kerala Agricultural University, neem oil and neem garlic emulsion at two per cent concentration were found to be effective for suppressing the population of *T. ludeni* on cowpea under field conditions. However, the mite population increased after five days which showed that these botanicals should be applied repeatedly for effective management of mites (Abhilash, 2001).

Patil (2005) evaluated the efficacy of various botanicals against *T. macfarlanei* on brinjal and observed that neem oil caused maximum egg hatch inhibition of 55.18 per cent. Neem oil also recorded significantly higher mortality of adults (21.67%) at 24 hours, which was on par with NSKE (18.33%).

Kumar (2007) reported that neem oil two per cent was effective in managing the mite population on rose cultivated under polyhouse condition. Leaves treated with neem oil two per cent recorded a lesser mean population of 3.81 mites/ leaf let as compared to the contol which had 23.77 mites/leaf. NSKE five per cent was moderately effective in managing the mite population on rose.

Babu *et al.* (2008) evaluated the effect of neem kernel aqueous extract (NKAE) against the red spider mite, *Oligonychus coffeae* (Nietner) (Acarina: Tetranychidae) infesting tea, both under laboratory and field conditions. The results

indicated that NKAE at 5 per cent concentration was effective against red spider mite, causing a high mortality of 83.43 per cent in laboratory bioassay. While in the field experiment, it recorded a lesser mean population of 272 mites/ 75 leaves as compared to the control which had 973 mites/ 75 leaves at two weeks after the third spray.

Patil and Nandhihalli (2009) studied the effect of promising botanicals against red spider mite on brinjal under field conditions and reported that neem oil two per cent exhibited maximum acaricidal action causing decrease in population of eggs (3.91/4 cm^2), immature active stages (3.81/4 cm^2) and adults of red spider mite (2.11/4 cm^2) at two days after first spray. Among various botanicals evaluated against spider mites on okra at Varanasi, NSKE five per cent caused maximum reduction (74.51%) followed by azadirachtin (67.27%) after 3 days of spraying (AINPAA, 2009).

Bernardi *et al.* (2012) reported that azadirachtin could cause reduction in population of *T.urticae* on strawberry ranging from 72 to 79 per cent at 7 days after spraying. Tehri and Gulati (2014) tested field efficacy of some biorationals against two spotted spider mite *T. urticae* and showed that nimbecidine (5 ml/l) caused 66.89 per cent reduction in mite population at fourth round of foliar application at an interval of 12 days

Marcic and Medo (2015) reported that azadirachtin significantly reduced gross fecundity, net fecundity and female longevity of red spider mite *T. urticae* at all different concentrations tested.

Krishna and Bhaskar (2016) conducted a field study to evaluate the botanicals, neem oil (2%) and NSKE (5%) against *T.urticae* on okra during two seasons. Neem oil recorded a significantly higher reduction in mite population of 81.15 per cent as against NSKE (52.78%) during the second season, though were on par with each other during the first season of study.

Sarmah (2016) evaluated the bioefficacy of neem kernel aqueous extract (NKAE) against tea red spider mite, *Oligonychus coffeae* both under laboratory and field conditions. The results revealed that NKAE at higher concentrations (6-10%) showed 53 to 95 per cent mortality of mite population under laboratory conditions and 43 to 69 per cent reduction of mite population under field conditions.

2.1.4 Entomopathogenic fungi for management of spider mites

Among the various entomopathogens employed against different crop pests throughout the world, fungi are considered as important in decimating mite population (Lipa, 1971). However only few entomopathogenic fungi have been commercially exploited in mite management. Fungi belonging to Hyphomycetes (Deuteromycota), including *Beauveria*, *Metarhizium*, *Paecilomyces* and *Verticillium* are some of them that have been observed on mites (Chandler, 2000).

Dresner (1949) reported that field application of *Beauveria bassiana* dust formulation containing 0.5 per cent conidia for the management of *T. urticae* recorded a mortality of 71.00 per cent and was one of the earliest evaluation of a fungus against Phytophagous mite.

The fungus *Hirsutella thompsonii* was first reported as a mite pathogen on the citrus rust mite, *Phyllocoptruta oleivora* in Florida where it was found to be causing epizootics in the mite population (McCoy and Kanavel, 1969). The moniliaceous fungus, *H. thompsoni* was reported to be highly pathogenic to the carmine spider mite, *Tetranychus cinnabarinus*, as well as to the oriental spider mite, *Eutetranychus orientalis*. The fungus penetrated the mite's integument mainly through the legs and formed hyphal bodies in haemolymph. Hyphae, on which the spores were produced, began to emerge through the anal and genital apertures and spread all over the body. The fungus killed most mites by the second day at 25°, 27° and 30° C but was not effective at both 13° and 35°C (Gerson *et al.*, 1979).

Gardner *et al.* (1982) carried out laboratory bioassay to determine the susceptibility of *T. urticae* to *H. thompsonii* by direct placement of conidia on mites

confined to bean leaf discs floating on distilled water and recorded a mean mortality of 96.5 per cent on 3 to 5 days after exposure to the conidia.

Rath (1991) studied the effect of the fungal pathogen *Metarhizium anisopliae* against citrus red mite, *P. oleivora* and reported the same to be promising as a biocontrol agent. The tetrapeptides and peptides isolated from the fungi *M. anisopliae and Tolypocladium niveum* showed toxic activity against *T.urticae* (Krasnoff *et al*., 1991).

Andreeva and Shternishis (1995) studied the effect of *H. thompsonii* on nymphs and adults of carmine spider mite and recorded 84 per cent infection of both stages five days after treatment. Pena *et al*. (1996) evaluated the effect of *B. bassiana* and *P. fumosoroseus* against *Polyphagotorsonemus latus* Banks and found *B. Bassiana* to be superior with a lower mean population of 8 mites/leaf as compared to 46 mites/leaf in case of *P. fumosoroseus* 9 days after spraying. Both cassava green mite, *M. tanajoa* and cotton red mite, *Oligonychus gossypii* (Zachner) were reported to be infected by *H. thompsoni* in the field in West Africa (Yaninek *et al*., 1996).

Tamai *et al*. (1999) reported that *Verticillium lecanii* and *B. bassiana* caused 100 per cent mortality of *T. urticae* at seven days after treatment. Tamai *et al*. (2002) observed 73 per cent mycosis on *T. urticae* within 5 days of inoculation with *Hirsutella* sp. at 1.7×10^7 conidia ml^{-1}.

Hanchinal and Manjunatha (2000) conducted pathogenicity study of *M. anisopliae* on vegetable mite *Tetranychus neocaledonicus* under laboratory conditions and recorded mortality of 92.89 per cent at a concentration of 1.5×10^8 spores ml^{-1} seven days after treatment. *Paecilomyces fumosoroseus* applied at the rate of 16.6 g l^{-1} was reported as effective in reducing the population of *Oligonychus coffeae* (Nietner) in a laboratory bioassay (Ramarethinam *et al*., 2000). The mortality ranged from 58.2 to 64.83 per cent on 10^{th} day after spraying and 75.68 to 95.68 on the 15^{th} day after spraying. Studies on diseases of mites caused by entomopathogenic fungi in Poland reported that the pathogenic fungi identified

were common representatives of the genus *Hirsutella* followed by *Neozygites floridana* and *Tarichium* (Mietkiewski *et al*., 2000).

Xian *et al*. (2001) observed that *T. urticae* population on green house vegetable crops was reduced by 70-80 per cent with the application of *V. lecanii* along with Tween 80 WP at the rate of 0.05 per cent. Nugroha and Ibrahim (2004) conducted a laboratory bioassay of three entomopathogenic fungi, *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* against broad mite, *Polyphagotarsonemus latus* Banks. *B. bassiana* recorded 80.88 per cent mortality at 1 x 10^8 conidia ml^{-1} while *M. anisopliae* and *P. fumosoroseus* caused 60 and 90 per cent mortality respectively. None of the three fungi had any significant ovicidal effect against this mite.

Wekesa *et al*. (2005) conducted pathogenicity test on adults of *T. evansi* with seventeen isolates of *M. anisopliae* and two isolates of *B. bassiana* and reported that *B. bassiana* was more pathogenic. The lethal mortality time of the same varied from 4.6 to 5.8 days. A reduction in the population to the tune of 98 per cent was recorded on nymphs and adults of *T. urticae* infesting tomato under glass house conditions by using commercial formulation of *B. bassiana* (Naturalis L.) (Chandler *et al*., 2005)

Prasanna (2007) reported that among different mycopathogens tested against *Tetranychus* sp. in brinjal ecosystem, *M. anisopliae* at 1.6 × 10^6 spores ml^{-1} recorded considerable reduction in mite population. Kalmath *et al*. (2007) conducted a survey in Karnataka during 2004 – 2005 and reported natural occurrence of fungal pathogens on tetranychid mites in tomato, beans and okra. An infection rate of 12.94 per cent by *B. bassiana* isolate was recorded on beans.

Tetranychus urticae population declined rapidly in green house cultivated bean plants on sixth day after second spray of *B. bassiana* which was statistically superior to *P. fumosoroseus* (Kalmath *et al*., 2008). A study on twelve entomopathogenic fungi for controlling broad mite *P. latus* in mulberry found that *M. anisopliae* isolate CKM-048 was the most virulent strain in controlling both

larvae and adults of broad mites at the concentration of 2×10^8conidia ml^{-1}. However, the strain did not show any ovicidal effect (Maketon *et al*., 2008).

Naik and Shekharappa (2009) conducted *in vitro* study on the bioefficacy of entomopathogenic fungal formulations against spider mites on okra. *M. anisopliae* recorded significantly higher mortality of 96.67 and 94.67 per cent in oil and wettable powder formulations repectively, followed by *B. bassiana* oil formulation (94.00 %).

Rachana *et al*. (2009) recorded 69.53 per cent reduction in the population of red spider mite, *T. neocaledonicus* on okra after spraying *Fuasrium semitectum* at the rate of 2.1×10^9 spores ml^{-1} along with *H. thompsonii* 4.6×10^8spores ml^{-1} and econeem (0.002%).

An *in vitro* bioassay was conducted with different isolates of the entomopathogenic fungus *B. bassiana* against *T. urticae*. *B. bassiana* isolates 444 Bb and 445 Bb had lethal effect even at the concentration of 10^6 conidia ml^{-1}. The mean mortality values of host individuals were 83.78 ± 3.62 per cent and 68.49 ± 4.28 per cent respectively one day after treatment and reached 100 per cent for both variants on the fourth day (Draganova and Simova, 2010).

Laboratory experiment was conducted on mite infested excised leaves of tea in order to explore the possibilities of exploiting local entomopathogenic fungal strain of *Hirsutella thompsoni*. The fungal strain *H. thompsoni* HF1 caused 65 per cent mortality but the highest mortality of 75 per cent was achieved by the recommended acaricide, Propargite (Amarasena *et al*., 2011).

Natural occurrence of two entomopathogenic fungi, *Cladosporium cladosporioides* (Fresenius) de Vries and *B. bassiana* were recorded on *T. urticae* infesting cowpea, red gram and okra in Coimbatore. Pathogenicity studies of the fungal isolates revealed that *C. cladosporioides* was more effective, recording 96.75 per cent mortality as compared to *B. bassiana,* which caused only 5.25 per cent mortality of mites (Jeyarani *et al*., 2011).

Seyed – Talebi *et al.* (2012) studied effects of *B. bassiana* on life parameters of *T. urticae* feeding on bean and cucumber under laboratory conditions. The fungus treated mites showed significantly lower adult longevity, oviposition period and fecundity.

Naturalis, a commercial formulation of *B. bassiana* was evaluated against *T. urticae* infesting vegetables grown under green house conditions. A reduction in mite population density by 86 and 93 per cent were recorded on cucumber and tomato respectively, when the mycopesticide was applied at 0.1 per cent concentration (Marcic *et al.*, 2012).

An anamorphic entomopathogenic fungus *Neozygites* sp. belonging to the family Neozygitaceae was found infecting the tetranychid mite. *T. urticae* on French bean (*Phaseolus vulgaris* L.) for the first time in Eastern Plateau and Hill region of India in the month of October, 2011 (Maurya *et al.*, 2013).

The pathogenicity of three entomopathogenic fungal species to *Tetranychus kanzawai* was investigated using seven isolates of *M. anisopliae*, six isolates of *B. bassiana*, and an isolate of *Paecilomyces lilacinus*. *M. anisopliae* Ma6 was found to be the most virulent isolate based on LC_{50} (5.0 $x10^2$) and LT_{50} (3.00 days). However, all *Metarhizium* and *Beauveria* isolates tested in the study showed potential for management of *T. kanzawai* (Sanjaya *et al.*, 2013).

Tehri and Gulati (2014) observed that *B. bassiana* at the rate of 1×10^{10} spores ml^{-1} significantly the reduced the population of *T. urticae* on cucumber (0.78 mites/cm^2) was compared to untreated control (9.38 mites/cm^2) under field conditions.

2.1.5 Management of spider mites by using *Acremonium zeylanicum*

Acremonium zeylanicum was first reported as an entomopathogenic fungus from India on *Aphis brassicae* Linn. by Chowdhary and Varshney (1983). They also described the morphological characters of *A. zeylanicum*. The fungal colonies grown on PDA medium attained 10 mm diameter in ten days at 20°C. Sporulation

was abundant. Phialides were simple, arising from aerial hyphae, 1.2 µm size, 7.25 µm long, tapering from 22.25 µ to 1 µm, thin walled, slender, smooth and hyaline. Conidia cohering in long chains, narrow oval, 3.6 x 1.25 – 2.5 µ in size, both ends truncate and acute and chlamydospores absent. The colonies of *A. zeylanicum* were moderately rapid growing and maturing in 4 to 7 days at 25°C. The colony was compact, white in colour and cottony due to overgrowth of fungus.

Steenberg and Humber (1999) isolated and bioassayed the entomopathogenic potential of *Verticillium* and *Acremonium* spp. against *Bemisia tabaci*, *Musca domestica* and *Alphitobius diaperinus*. *Acremonium* sp. caused mortalities that differed significantly from those in the control.

Acremonium colony growth rate was moderately rapid and matured within 5 days. The diameter of the colony was 1 to 3 cm when grown on potato dextrose agar and incubated at 25°C for 7 days. The texture of the colony was compact occasionally raised in the centre. By ageing, the surface of the colony might become cottony due to the over growth of hyphae. The colour of the colony was white, pale pink on the lower surface and it was either hyaline or cream coloured in pigmentation at the reverse side (Kulkarni *et al*., 2006). Natural incidence of *A. zeylanicum was* later reported on sugarcane wooly aphid from northern Karnataka (Tippannavar *et al*., 2006).

Naveenkumar (2007) evaluated *A. zeylanicum* against sugarcane wooly aphid under laboratory conditions and reported that the first instar nymphs of SWA recorded highest mortality 92.50 per cent at 1 x 10^{10} conidia l^{-1} concentration of the fungus. As the stage of instar advanced, the mortality rate declined to 88.50, 84.0 and 83.30 per cent for II, III and IV instar, respectively. Lower mortality of aphids was recorded at 1 x 10^{4} conidia l^{-1} 10 days after application.

Divan and Mallapur (2011) evaluated the pathogenicity of the fungus *A. zeylanicum* to different aphid species and it was found to be highly pathogenic to sorghum aphid (*Melanaphis sacchari* Zehnt.), cabbage aphid (*Brevicoryne brassicae* Linn.) and sugarcane wooly aphid (*Ceratovacuna lanigera* Zehnt.) all of

which recorded more than 60 per cent mortality 10 days after spraying at all tested concentrations.

Shalini *et al.* (2011) reported *A. zeylanicum* to cause mortality of cotton mirid bug (*Creontiades biseratence* Distant.) to the tune of 69.05, 78.57 and 86.24 per cent at concentrations of 1×10^7, 1×10^8 and 1×10^9 conidia ml^{-1} respectively under laboratory conditions.

During 2012, large scale mycosis of *T. urticae* was noticed on brinjal in polyhouse of Thrissur district, Kerala and three fungal pathogens *viz.*, *Neozygites floridana*, *A. zeylanicum* and *Conidiobolus* sp. were identified from these mycozed mites. Studies on the efficacy of these fungi against *T. urticae* are to be conducted under polyhouse and open field conditions to identify their suitability as potent candidates in biological control programme (Krishna *et al.*, 2014).

2.2 Sensitivity of fungi to different agrochemicals

Anderson *et al.* (1989) studied the effects of comibination of *B. bassiana* with different insecticides for the management of colorado potatao beetle and the results revealed that abamectin, triflumuron and thuringiensin at sublethal doses were compatible with *B. bassiana* as no significant inhibition of *B. bassiana* either germination or growth was detected.

A study was conducted to evaluate the compatibility of entomopathogenic fungus *B. bassiana* with 12 acaricide formulations under laboratory conditions. It was found that the different acaricides formulations affected vegetative growth, conidial germination and sporulation of the *B. bassiana* to different levels. The formulations most compatible with *B. bassiana* were avermectin and pyrethroids and the remaining tested acaricides like chlorfenapyr, fenpyroximate, amitraz, acrinathrin, hexythiazox, abamectin, pyridine, dimethoate, pyridaphethion, fenbutatin oxide, azocyclotin and cyhexatin affected conidial germination, vegetative growth and sporulation of the fungus (Oliveira and Neves, 2004).

Thirty isolates of *B. bassiana* were screened with a commercial formulation of neem oil and 23 isolates were found compatible with neem oil. All the 23 isolates, in combination with the commercial formulation neem was found to have synergistic effect on insect mortality. Combined treatment of neem with neem sensitive isolates showed an antagonistic effect on insect mortality (Mohan *et al.*, 2007)

Rachappa *et al.* (2007) studied the effect of different agrochemicals like fungicides, insecticides and weedicides on growth and sporulation of *M. anisopliae*. The fungicides were the most inhibitory (78.18%) to the fungus followed by insecticides (44.23%) and weedicides (20.33%). Among the different insecticides group, 60.69 per cent inhibition of the fungus was recorded by chlorinated hydrocarbons (endosulfan and dicofol) followed by organophosphates (46.66% inhibition) and carbamates (45.45% inhibition). On the other hand, imidacloprid (11.10% inhibition) and spinosad (5.10 %) were found to be safe to the fungus.

Conventional insecticide products used for control of sweetpotato whitefly, *Bemisia tabaci* were evaluated against entomopathogenic fungus *Lecanicillium muscarium* (Andrew *et al.*, 2008). Among the different insecticides tested, majestic (natural plant extract), spray oil (petroleum oil) and savona (fatty acids) resulted in very low spore germination following direct exposure for 24 h. However the chemicals oberon (spiromesifen) and agri 50E (polysaccharide) produced acceptable spore germination.

Prasanna and Mallapur (2011) carried out an *in vitro* evaluation to assess the sensitivity of *A. zeylanicum* to different chemicals. They found that the fungus *A. zeylanicum* was highly sensitive to the pyrethroid, cypermethrin (73.12% growth inhibition) which was followed by the organochlorines dicifol and endosulfan as well as the organophosphate, chlorpyriphos. The lowest inhibition of 29.58 and 30.64 per cent were recorded by the thiamethoxam and imidacloprid respectively. Fungicides inhibited the growth of this fungus by 62.38 to 83.35 per cent.

Compatibility of entomopathogenic fungi like *B. bassiana*, *P. fumosorosea* and *L. lecanii* with botanicals were assesed and the results showed that commercial botanical formulations like biospark, phytophrate and exodos did reduced the mycelial growth of the entomopathogenic fungi examined (Sahyaraj *et al*., 2011).

Akbar *et al*. (2012) conducted a study to assess the toxicity of insecticides and fungicides to spore production and mycelial growth of *M. anisopliae*. Among the different chemicals tested chlorpyriphos, lufenuron, profenofos and metalaxyl+mancozeb were the most toxic to mycelial growth and conidial germination followed by emamectin, cypermethrin, acetameprid, imidacloprid and sinophos which were relatively less toxic. Spinosad and indoxacarb were found to be the most compatible insecticides.

Amjad *et al*. (2012) carried out an *in vitro* experiment using nine commercial pesticides against *Isaria fumosorosea* and *Lecanicillium muscarium*. All pesticides inhibited conidial germination as well as mycelial growth significantly. Azocyclotin was proven to be the most toxic followed by pyridaben, acetamiprid and propargite, while buprofezin was reported to be least toxic to the germination of spores as well as mycelial growth. According to them the germination of conidia was more sensitive than mycelial growth of both the fungi.

The effect of fipronil, pyriproxyfen and hexaflumuron on sporulation, vegetative growth and conidial germination of *M. anisopliae* was studied based on measuring the vegetative growth and sporulation (Rashid *et al*., 2010). Hexaflumuron completely inhibited the germination of spores while fipronil and pyriproxyfen recorded 32.36 and 9.70 per cent germination. At highest concentration of pesticides tested, hexaflumuron, fipronil and pyriproxyfen caused 100, 76.6 and 76.4 per cent reduction in vegetative growth respectively.

2.3 Safety of entomopathogens to the predatory mite

Studies pertaining to the safety of entomopathogen on predatory mite *Neoseiulus longispinosus* are scanty. Hence related study on other predatory mites are reviewed hereunder.

B. bassiana recorded more than 10 per cent mortality of *N. californicus* under laboratory conditions (Castagnoli *et al.*, 2005). Duso *et al.* (2008) conducted a laboratory study to compare the toxicity of *B. bassiana* to Mediterranean population of *T. urticae* and *Phytoseiulus persimilis*. They reported that *B. bassiana* resulted in mortality of 43 per cent and egg hatchability of 98.13 per cent on *Phytoseiulus persimilis*. Mortality rate of *P. persimilis* and *N. californicus* was 43 and 14 per cent respectively when tested at the highest concentration of *B. bassiana* (1.25×10^7 conidia ml^{-1}) and found that the fungus was more pathogenic to *P. persimilis* than *N. californicus* (Vergel *et al.*, 2011).

Wu *et al.* (2014) worked out the effect of the selected strain SZ-26 of *B. bassiana* on the predatory mite *Neoseiulus (Amblyseius) barkeri* Hughes. They recorded that the preoviposition duration of predator was significantly longer as compared to the control and there were no differences in other life table parameters, such as oviposition, postoviposition duration, female longevity and daily fecundity compared to the control.

Material & Methods

3. MATERIAL AND METHODS

The present study was carried out in the Acarology laboratory, Department of Agricultural Entomology, College of Horticulture, Vellanikkara during 2015-2017. The objectives of the investigation were to evaluate the biocontrol potential of *Acremonium zeylanicum* (Petch) Gams and Evans against the spider mite, *Tetranychus truncatus* Ehara, to assess the sensitivity of the acaropathogen to selected novel acaricides and botanicals and also to evaluate safety of *A. zeylanicum* to the predatory mite, *Neoseiulus longispinosus* (Evans).

The materials used and methods adopted for conducting various experiments to fulfill the objectives in the study are elaborated hereunder.

3.1 Evaluation of the biocontrol potential of *Acremonium zeylanicum* against the spider mite, *Tetranychus truncatus*

3.1.1 Mass culture of *Tetranychus truncatus*

Tetranychus truncatus was mass cultured in the laboratory on mulberry leaves placed in plastic trays (40×28 cm^2) lined with moistened synthetic absorbent sponge (Plate 1). Mulberry leaves were placed upside down on wet sponge and gravid females collected from the nuclear culture maintained in the Acarology laboratory by AINPAA (All India Network Project on Agricultural Entomology) were released on the leaves. Leaves were replaced with fresh ones once every four days. For replacing mulberry leaf, old leaf was placed above the new leaf so that *T. truncatus* got transferred to the new leaf naturally.

T. truncatus culture was also maintained on three weeks old potted okra plants (variety *Arka Anamika*) in the polyhouse at AINPAA, Department of Agricultural Entomology (Plate 2). Mite culture was initiated by releasing laboratory reared *T. truncatus* onto the leaf of okra plant. The potted plants were replanted periodically to reduce the effect of plant age on mite development and fecundity.

Plate 1. Laboratory culture of *Tetranychus truncatus* on mulberry leaves

Plate 2. Mass culturing of *Tetranychus truncatus* on potted okra plants in polyhouse

3.1.2 Maintenance of *Acremonium zeylanicum* culture

The pure culture of the acaropathogen, *A. zeylanicum* procured from AINPAA laboratory was used for the study (Plate 3) and was subcultured on specific medium, Sabouraud Dextrose Agar with the addition of 2 per cent Yeast extract (SDAY) (Appendix 1) in Petri dishes. To maintain the virulence, it was inoculated on *T. truncatus* after 5 to 6 rounds of subculturing. For this, the fungus was reisolated from moribund mites on SDAY medium. The medium was sterilized in an autoclave at 121°C temperature and 15 PSI pressure for 20 minutes. After sterilization, the medium was cooled and transferred approximately 20 ml to sterilized Petri dishes and the dishes were inoculated with pure culture of fungus under aseptic condition. All the plates were incubated at room temperature and observed for fungal growth next day onwards. The purity of the culture was confirmed by observing the culture under a microscope by preparing slides. The pure culture was transferred to SDAY slants for further work.

3.1.3 Evaluation of *A. zeylanicum* in laboratory

Laboratory bioassays were conducted separately on egg and gravid female of *T. truncatus* to evaluate the bioefficacy of *A. zeylanicum* at five different concentrations namely 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 spores ml^{-1}. Broth culture of *A. zeylanicum* in SDAY medium with thick fungal mat maintained in the laboratory was ground thoroughly in an ordinary sterilized mixer to prepare spore suspension. The suspension was shaken thoroughly with a drop of 0.05 per cent Tween 80 for uniform dispersion of spores. A suspension of spores was filtered through a double layered muslin cloth and spore count was made using Neubauers haemocyotmeter under a phase contrast microscope. From the stock solution, further dilutions were made to obtain the required concentrations.

The spore concentration was calculated using the formula,

$$\text{Number of spores ml}^{-1} = \frac{X \times 25 \times 10 \times 1000 \times D}{Y}$$

Plate 3. *Acremonium zeylanicum* culture

Plate 3a. SDAY slant

Plate 3b. SDY broth

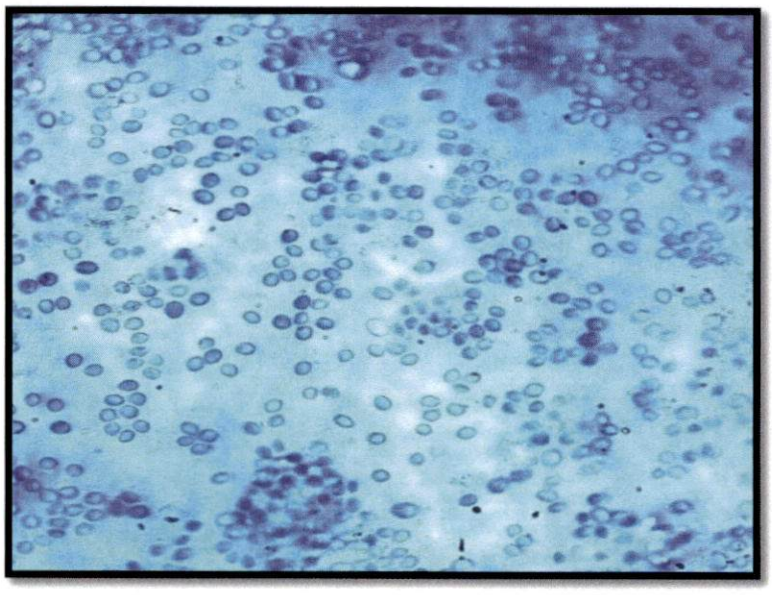

Plate 3c. Spores of *Acremonium zeylanicum* (100 X)

Where,

X = Number of spores counted totally in big square

Y = Number of big squares counted

D = Dilution factor

3.1.3.1 Ovicidal effect

Ovicidal bioassay was conducted on *T. truncatus* using topical application method with five different concentrations of the acaropathogen *A. zeylanicum*.

To evaluate the ovicidal effect, eggs of uniform age were obtained by transferring eight gravid females each of *T. truncatus* from laboratory culture; using moistened zero size camel hair brush on to three mulberry leaf bits (5×5 cm^2) placed in Petri plates lined with moistened cotton pad. The females were removed after 24 h. Twenty five eggs were retained per leaf bit after removing the excess eggs. Leaf bits containing *T. truncatus* eggs were sprayed with required concentration of treatments to be tested using a hand atomizer (Plate 4). The treated leaf bits with eggs were dried at room temperature for 1 h and placed in Petri plates. All treatments were replicated three times (Plate 5). Egg mortality was based upon the distortion or shrinkage in egg shape and non-emergence. Observations on mortality of eggs were recorded at 24, 48, 72 and 96 h interval under a stereo binocular microscope.

3.1.3.2 Adulticidal effect on gravid females

For studying the effect of different treatments on gravid females, aqueous preparation of the fungal isolate at required concentrations were made by serial dilution. The treatments were applied using a hand atomizer on mulberry leaves kept on wet cotton pad in Petri plates separately and allowed to dry for 2 h. Twenty five gravid females of *T. truncatus* of uniform age taken from the laboratory culture were released on each treated leaf. Leaf treated with sterile water served as control (Plate 6). Observations on mortality of mites were

Plate 4. Topical application of treatment

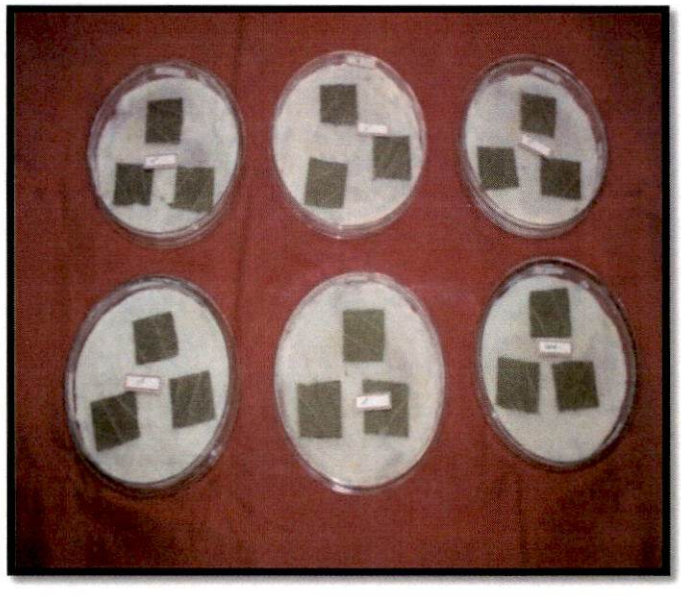

Plate 5. Bioassay on eggs of *Tetranychus truncatus*

Plate 6. Bioassay on adults of *Tetranychus truncatus*

Plate 7. Layout of experiment in polyhouse on cucumber

recorded at 24 h interval for 7 days under a stereo binocular microscope and per cent mortality was calculated. Pathogenicity of the fungus *A. zeylanicum* on *T. truncatus* was proved using Koch postulates.

3.1.3.3 Data analysis

The data were converted to per cent mortality and then transformed into arcsine values for computation of analysis of variance. Per cent mortality data was corrected with the control mortality by using Abbott's formula.

3.1.4 Evaluation of *A. zeylanicum* in polyhouse

A field experiment was conducted to evaluate the efficacy of acaropathogenic fungi, *A. zeylanicum* at two different concentrations along with two newer acaricide molecules viz., spiromesifen and diafenthiuron and two botanicals *viz*., neem oil and azadirachtin and an untreated control (Table 1) against the *T. truncatus* on cucumber (Variety Sania) in the polyhouse of AINPAA during March to May, 2017. The crop was raised in the polyhouse as per the Package of Practices Recommendations (KAU, 2016) at a spacing of 60×30 cm in plots of 1.6 m × 1.3 m size (Plate 7). The experiment was laid out in Completely Randomized Design with seven treatments and three replications.

Mites were released on three leaves of twenty five days old cucumber plant at the rate of 20 active mites/leaf by stapling mite infested mulberry leaf bits of 5 cm^2 size on top, middle and bottom leaf of cucumber plant.

Treatments were imposed three weeks after the release of mites. Spray solution was prepared by thorough mixing of measured quantity of different treatments and required amount of water to form a uniform suspension. The treatments were applied using a hand sprayer. A control treatment was maintained with water spray. Observations were recorded on eggs and active stages of *T. truncatus* from three windows of 1 cm^2 each from three leaves per plant representing the top, middle and bottom canopy. The population counts were

recorded one day before spraying and 1st, 3rd, 7th, 10th and 14th day after spraying, for which number of mites/cm^2 leaf area was recorded *in situ* using a hand lens of 10 X magnification (Plate 8.). To confirm the results, the experiment was repeated in the existing crop. As the population of mite was found to be negligible in all treatments except control following the first round of spray itself, a second release of the mite was made and two weeks after the release the treatments were imposed. Observations were recorded in a similar manner on one day before spraying and 1st, 3rd, 7th, 10th and 14th day after spraying. The mean mite count was worked out and analyzed statistically.

3.1.4.1 Statistical analysis and interpretation of data

Data on mean population of mites on one, three, seven, ten and fourteen days after treatment application were tested by analysis of covariance (ANOCOVA), taking population counts prior to the first application as covariate. The result obtained was subjected to DMRT (Duncan's Multiple Range Test). The mean per cent reduction in population over precount of mites was also worked out at seven and fourteen days after treatment application.

Table 1. Treatments evaluated against red spider mite *T. truncatus* on cucumber

Sl. No.	Treatments	Dosage	Trade name
1	*Acremonium zeylanicum*	1×10^7 spores ml^{-1}	Local isolate
2	*Acremonium zeylanicum*	1×10^8 spores ml^{-1}	Local isolate
3	Spiromesifen	100g ai ha^{-1}	Oberon 240 SC
4	Diafenthiuron	400g ai ha^{-1}	Pegasus 50 WP
5	Neem oil 2%	20 ml l^{-1}	Neem oil
6	Azadirachtin 0.005%	5 ml l^{-1}	Econeem plus
7	Untreated control	-	-

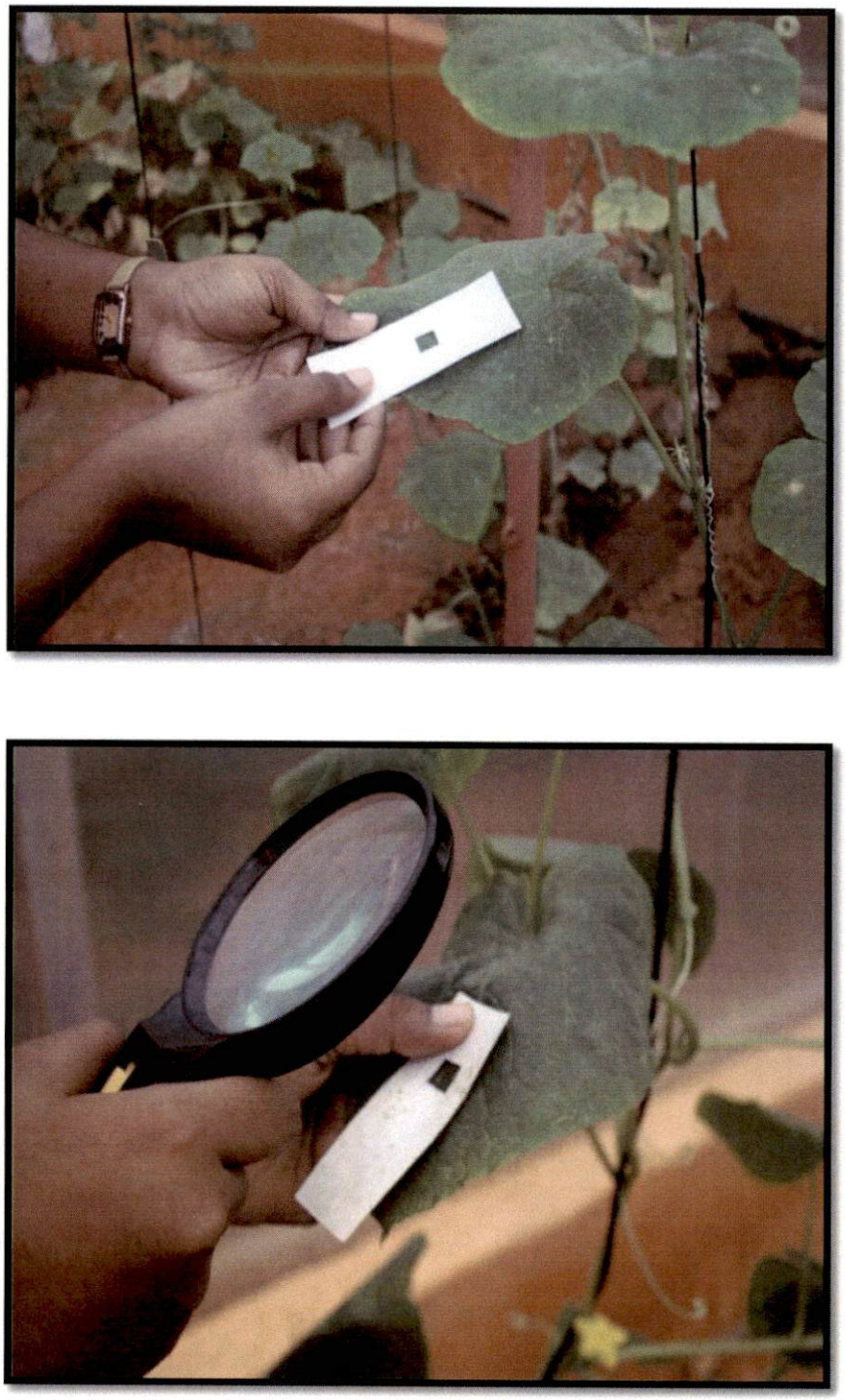

Plate 8. ***In situ*** **counting of mites**

3.2 Assessment of sensitivity of *A. zeylanicum* to different agrochemicals / botanicals

The compatibility of *A. zeylanicum* with selected agrochemicals was assessed following poisoned food technique. Details of the different acaricides, botanicals and fungicides used in the experiment are furnished in Table 2. The effect of these chemicals on the radial growth and germination of *A. zeylanicum* was evaluated. SDAY media (60 ml) was autoclaved at 121°C for 15 minutes, allowed to cool and then added with 0.3 g l^{-1} of streptomycin sulphate. Requisite quantity of acaricides, botanicals and fungicides were added to the SDAY medium in flask and mixed thoroughly before solidification to get the desired concentration. The medium was then poured equally into three Petri plates. Small disc (5 mm dia) of the fungal mycelium was cut with sterile cork borer and placed aseptically at the centre of the plate containing poisoned medium and the plates were sealed with parafilm. Each treatment was replicated thrice and the growth medium (SDAY) without insecticide but inoculated with mycelial disc served as untreated check. Inoculated plates were incubated at room temperature. Observations on colony diameter were recorded next day onwards till the control plates showed 90 mm growth and the per cent inhibition was calculated as described by Vincent (1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Diameter of fungal growth in control

T = Diameter of fungal growth in treatment

Table 2. Details of different agrochemicals used in the experiment

Sl. No.	Common name	Trade name	Concentration tested
	Acaricides		
1	Spiromesifen	Oberon 240 SC	0.7 ml l^{-1}
2	Diafenthiuron	Pegasus 50 WP	1.6 g l^{-1}
3	Fenazaquin	Magister 10 EC	2.5 ml l^{-1}
4	Fenpyroximate	Sedna 5 SC	1 ml l^{-1}
5	Propargite	Omite 57 EC	1.5 ml l^{-1}
	Botanicals		
6	Neem oil 2 %	Neem oil	20 ml l^{-1}
7	Azadirachtin 0.005 %	Econeem plus	5 ml l^{-1}
	Fungicides		
8	Cymoxanil 8% + Mancozeb 64%	Curzate M8	2 g l^{-1}
9	Famoxadone 16.6%+Cymoxanil 22.1%	Equation pro	1 ml l^{-1}
10	Untreated control	-	-

3.3 Evaluation of safety of *A. zeylanicum* to the predatory mite, *Neoseiulus longispinosus*

3.3.1 Laboratory Culture of *Neoseiulus longispinosus*

Neoseiulus longispinosus (Plate 9) was multiplied in the laboratory on *T. truncatus* infested mulberry leaves. The mulberry leaves were maintained on plastic trays (40×28 cm^2) lined with moistened synthetic absorbent sponge (Plate 10). Mulberry leaves were placed upside down on wet sponge and gravid females of *T. truncatus* were released. One week after the release of prey mite, five gravid females of *N. longispinosus* were released on the leaf. Predatory mite culture was monitored daily and prey mites were replenished periodically. Leaves were replaced with fresh ones once every four days. For replacing mulberry leaf, old leaf was placed above the new leaf so that the prey mite, *T. truncatus* and predatory mite, *N. longispinosus* got transferred to the new leaf naturally.

3.3.2 Laboratory bioassay

Laboratory bioassays were conducted separately on egg and active stage of *N. longispinosus* to evaluate the pathogenicity of *A. zeylanicum* at two best concentrations selected from the laboratory study namely 1×10^8 and 1×10^9 spores ml^{-1} along with a control.

3.3.2.1 Ovicidal effect

Two different concentrations of *A. zeylanicum* were topically applied on *N. longispinosus* eggs to evaluate the ovicidal effect.

To evaluate the ovicidal effect, eggs of uniform age of *N. longispinosus* were obtained by transferring four females from laboratory culture using moistened zero size camel hair brush on to *T. truncatus* infested mulberry leaves placed in Petri plates lined with moistened cotton pad. To prevent the escape of mites from the leaves in a Petri plate, a thin lining of wet cotton was provided all around the leaf margin. The females were removed after 24 h. Six eggs of predator were retained per leaf and the excess eggs were removed. Leaves

Plate 9. ***Neoseiulus longispinosus***

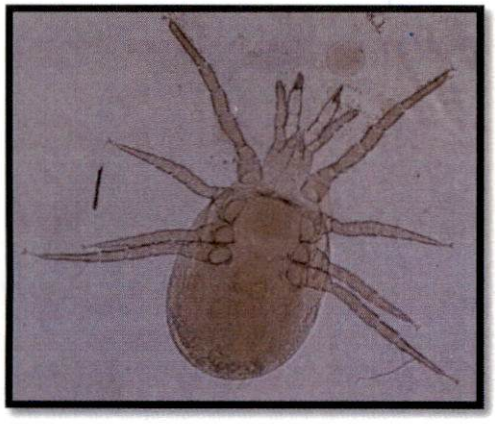

Plate 9a. Slide mounted view of *N. longispinosus*

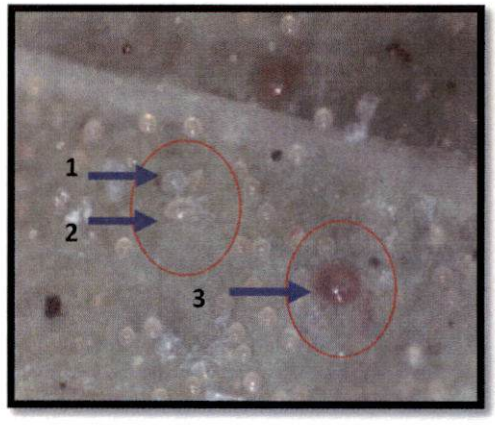

Plate 9b. egg(1), nymph(2) and adult(3) of *N. longispinosus*

Plate 9c. Nymphs of *N. longispinosus* targeting the adult prey *Tetranychus truncatus*

Plate 10. Culturing of *Neoseiulus longispinosus* on mulberry leaves in laboratory

Plate 11. Bioassay on eggs of *Neoseiulus longispinosus*

Plate 12. Bioassay on adults of *Neoseiulus longispinosus*

containing *N. longispinosus* eggs were sprayed with required concentration of treatments to be tested using a hand atomizer. The treated leaves along with the eggs were dried at room temperature for 1 h and placed in Petri plates. All treatments were replicated five times (Plate 11). The number of eggs hatched were recorded at 24, 48, 72 and 96 h of treatment under a stereo binocular microscope and mortality calculated.

3.3.2.2 Adulticidal effect on *N. longispinosus*

For studying the effect of different treatments on survival active stages of *N. longispinosus*, aqueous preparation of the fungal isolate at required concentrations were made by serial dilution. The treatments were applied using a hand atomizer on mulberry leaves kept on wet cotton pad on Petri plates separately and allowed to dry for two hours. Six *N. longispinosus* females of uniform age taken from the laboratory culture were released on each treated leaf with the aid of a fine brush. Leaf treated with sterile water served as control (Plate 12). To prevent the escape of mites from the leaves in the Petri plate, a thin lining of wet cotton was provided all around the leaf margin. Adequate number of different stages of *T. truncatus* were released on each treated leaves as prey for the predator. Fresh spider mites were added to each leaves daily to ensure an abundance of food. All treatments were replicated five times. Mortality of predatory mites were recorded at 24 h interval for seven days under a stereo binocular microscope. Moribund mites were considered as dead.

3.3.2.3. Data analysis

The data were converted to per cent mortality and then transformed into arcsine values for computation of analysis of variance.

Experimental Results

4. Results

A study was conducted in the Acarology laboratory, Department of Agricultural Entomology, College of Horticulture to evaluate the biocontrol potential of *Acremonium zeylanicum* (Petch) Gams and Evans against the spider mite, *Tetranychus truncatus* Ehara, to assess the sensitivity of the acaropathogen to selected novel acaricides and botanicals and also to evaluate safety of *A. zeylanicum* to the predatory mite, *Neoseiulus longispinosus* (Evans). The results of the study are presented in this chapter.

4.1 Evaluation of the biocontrol potential of *Acremonium zeylanicum* against the spider mite, *Tetranychus truncatus*

4.1.1. Evaluation of *A. zeylanicum* in laboratory

4.1.1.1 Ovicidal effect

The bioassay studies revealed that the eggs did not hatch for 48 h in any of the treatments evaluated including control. At 72 h, the highest egg hatchability (60.00%) was recorded in control treatment followed by *A. zeylanicum* at the rate of 1×10^5 spores ml^{-1} (50.66%) which were on par with each other. Treatment 1×10^6 spores ml^{-1} recorded 36.00 per cent hatchability and was on par with 1×10^5 spores ml^{-1}. The treatments 1×10^7 spores ml^{-1} and 1×10^8 spores ml^{-1} recorded hatchability of 26.67 and 22.67 per cent respectively and were on par with each other and also with the treatment 1×10^6 spores ml^{-1}. The lowest egg hatchability (9.33%) was recorded in the treatment 1×10^9 spores ml^{-1}. The hatchability increased 96 h after treatments, with the highest hatchability of 93.33 per cent being observed in the control. This was followed by the treatment 1×10^5 spores ml^{-1} (80.00%). The treatments 1×10^6, 1×10^7 and 1×10^8 spores ml^{-1} recorded hatchability of 69.33, 69.33 and 68.00 per cent respectively and were on par with the treatment 1×10^5

spores ml^{-1}. The treatment 1×10^9 spores ml^{-1} recorded a hatchability of 58.67 per cent at 96 h which was significantly lower than all other treatments (Table 3).

Per cent mortality of eggs at 96 h after treatment was also worked out. *A. zeylanicum* at 1×10^9 spores ml^{-1} recorded significantly higher mortality of 41.33 per cent. This was followed by 1×10^8 spores ml^{-1}(32.00%), 1×10^7 spores ml^{-1} (30.67%) and 1×10^8 spores ml^{-1} (30.67%) which were on par with each other. The treatment 1×10^5 spores ml^{-1} recorded a significantly lower mortality of 20.00 per cent. However, the lowest egg mortality of 6.66 per cent was recorded in the control treatment.

Table 3. Effect of *Acremonium zeylanicum* on eggs of *Tetranychus truncatus*

Sl. No.	Treatment No.	Treatment	Per cent hatchability				Per cent mortality
			24 h	48 h	72 h	96 h	96 h
1	T1	1×10^5 spores ml^{-1}	0.00 (0.573)	0.00 (0.573)	50.66 ^{ab} (45.382)	80.00^{b} (63.50)	20.00^{c} (26.492)
2	T2	1×10^6 spores ml^{-1}	0.00 (0.573)	0.00 (0.573)	36.00 ^{bc} (36.706)	69.33 ^{bc} (56.384)	30.67^{b} (33.616)
3	T3	1×10^7 spores ml^{-1}	0.00 (0.573)	0.00 (0.573)	26.67 ^{c} (30.923)	69.33 ^{bc} (56.411)	30.67^{b} (33.589)
4	T4	1×10^8 spores ml^{-1}	0.00 (0.573)	0.00 (0.573)	22.67 ^{c} (28.287)	68.00 ^{bc} (55.577)	32.00^{b} (34.423)
5	T5	1×10^9 spores ml^{-1}	0.00 (0.573)	0.00 (0.573)	9.33^{d} (14.806)	58.67^{c} (49.99)	41.33^{a} (40.00)
6	T6	Control	0.00 (0.57)	0.00 (0.57)	60.00 ^{a} (50.82)	93.33^{a} (77.57)	6.66^{d} (12.42)
CD value (p = 0.05)			NS	NS	12.56	8.44	4.05

Means followed by same letter in the column do not differ significantly by DMRT(P =.05); figures in parentheses are arcsine transformed values

4.1.1.2. Adulticidal effect on gravid females

The effect of *A. zeylanicum* on the gravid females of *T. truncatus* is furnished in Table 4. The data on pathogenicity of the fungus on mite at one day after treatment revealed no mortality in any of the treatments tested. However, in the treatments 1×10^9 spores ml^{-1} and 1×10^8 spores ml^{-1}, 27.05 and 17.14 per cent mites were found to be in moribund state respectively. There was no significant mortality in the treatments 1×10^5 spores ml^{-1}, 1×10^6 spores ml^{-1} and 1×10^7 spores ml^{-1} at two days after spraying even though the treatments 1×10^9 spores ml^{-1} and 1×10^8 spores ml^{-1} recorded 29.22 and 22.16 per cent mortality respectively. At three days after spraying, treatment 1×10^9 spores ml^{-1} recorded a highest mortality of 37.99 per cent and was on par with the treatment 1×10^8 spores ml^{-1} which recorded 31.64 per cent mortality. This was followed by 1×10^7 spores ml^{-1} which recorded 7.72 per cent mortality. At four days after spraying, the treatments 1×10^9 spores $ml^{-1}$and 1×10^8 spores ml^{-1} with 39.68 and 34.46 per cent mortality respectively were on par with each other. The treatment 1×10^7 spores ml^{-1} registered a mortality of 13.90 per cent and was on par with 1×10^8 spores ml^{-1}. This was followed by 1×10^6 spores ml^{-1} and 1×10^5 spores ml^{-1} with 8.70 and 7.05 per cent mortality respectively.

The treatment 1×10^9 spores ml^{-1} recorded the highest mortality of 45.59 and 58.73 per cent at five and six days after spraying respectively. At five days after spraying, the treatment 1×10^8 spores ml^{-1} recorded 38.70 per cent mortality and was followed by the treatments 1×10^7 spores ml^{-1} (20.36%), 1×10^6 spores ml^{-1} (12.32%) and 1×10^5 spores ml^{-1} (12.70%). More than 50 per cent mortality of mites was recorded within six days of spraying in treatments 1×10^9 spores ml^{-1} and 1×10^8 spores ml^{-1} (Plate 13). At six days after spraying, the treatment 1×10^7 spores ml^{-1} recorded 37.79 per cent mortality and was on par with the above treatments. The treatment 1×10^6 spores ml^{-1}, with 19.95 per cent mortality was on par with the treatment 1×10^7 spores ml^{-1}. The treatment 1×10^5 spores ml^{-1} failed to induce any

significant mortality of mites even seven days after spray. On the seventh day, 1×10^{9} spores ml^{-1} recorded the highest mortality of 68.36 per cent which was significantly superior to other treatments namely 1×10^{8} spores ml^{-1} (61.65%), 1×10^{7} spores ml^{-1} (52.00%), 1×10^{6} spores ml^{-1} (29.49%) and 1×10^{5} spores ml^{-1} (13.43%).

Table 4. Effect of *Acremonim zeylanicum* on gravid females of *Tetranychus truncatus*

Sl. No.	Treatment No.	Treatment	Per cent mortality of *T. truncatus*						
			1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
1	T1	1×10^5 spores ml^{-1}	-0.36 ^{b} (5.85)	-4.76 ^{b} (0.57)	-8.25 ^{c} (0.57)	7.05 ^{cd} (10.11)	12.70 ^{cd} (13.08)	12.90 ^{cd} (12.68)	13.43 ^{c} (12.83)
2	T2	1×10^6 spores ml^{-1}	-0.43 ^{b} (9.68)	1.50 ^{b} (4.48)	3.17 ^{bc} (6.37)	8.70 ^{cd} (14.86)	12.32 ^{bc} (19.16)	19.95 ^{bc} (26.10)	29.49 ^{b} (32.77)
3	T3	1×10^7 spores ml^{-1}	-0.97 ^{b} (7.13)	0.07 ^{b} (4.48)	7.72 ^{b} (13.40)	13.90 ^{bc} (21.21)	20.36 ^{ab} (33.00)	37.79 ^{ab} (37.80)	52.00 ^{ab} (46.21)
4	T4	1×10^8 spores ml^{-1}	17.14 ^{a} (24.06)	22.16 ^{a} (28.48)	31.64 ^{a} (34.20)	34.46 ^{ab} (35.92)	38.70 ^{a} (38.46)	56.16 ^{a} (48.64)	61.65 ^{a} (51.96)
5	T5	1×10^9 spores ml^{-1}	27.05 ^{a} (31.30)	29.22 ^{a} (32.71)	37.99 ^{a} (37.97)	39.68 ^{a} (39.02)	45.59 ^{a} (42.47)	58.73 ^{a} (50.12)	68.36 ^{a} (56.04)
6	T6	Control	0.00 ^{b} (0.573)	0.00 ^{b} (0.573)	0.00 ^{c} (0.573)	0.00 ^{d} (0.573)	0.00 ^{b} (0.573)	0.00 ^{d} (0.573)	0.00 ^{c} (0.573)
CD value (p=0.05)			13.06	8.25	11.75	17.10	17.82	17.77	18.64

Means followed by same letter in the column do not differ significantly by DMRT (P=.05), DAS - days after spraying; figures in parentheses are arcsine transformed values

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Plate 13. *Acremonium zeylanicum* infected *Tetranychus truncatus*

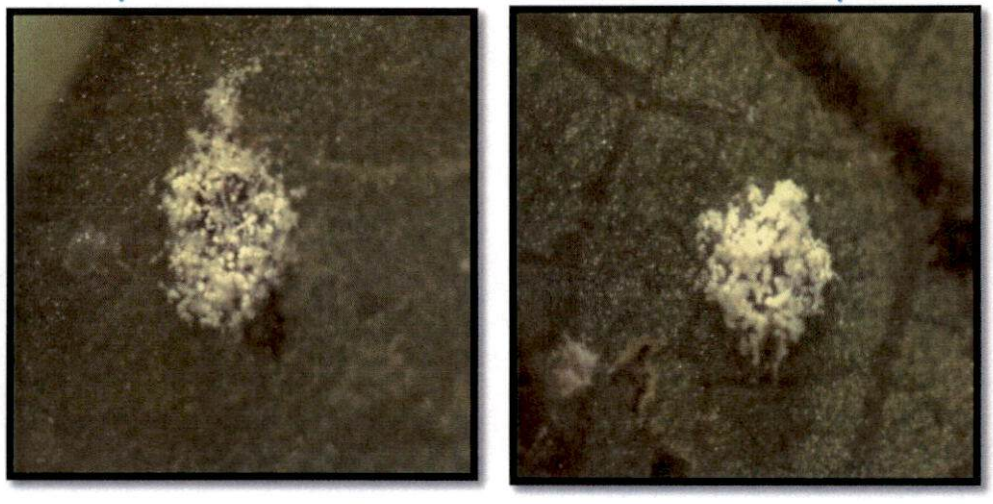

Plate 13a. Mycosed *Tetranychus truncatus* after treatment in laboratory (35 X)

Plate 13b. Mycosed *Tetranychus truncatus* after treatment in polyhouse (35 X)

4.1.2 Evaluation of *A. zeylanicum* in polyhouse

A field experiment was conducted to evaluate the efficacy of the acaropathogenic fungi, *A. zeylanicum* at two different concentrations along with two novel acaricide molecules *viz*., spiromesifen and diafenthiuron, two botanicals *viz*., neem oil and azadirachtin and an untreated control against *T. truncatus* on cucumber (Variety Sania) in the polyhouse of AINPAA, Department of Agricultural Entomology, College of Horticulture, Vellanikkara during the period from March to May, 2017.

4.1.2.1 Efficacy of treatments against *Tetranychus truncatus* on cucumber (First experiment)

The results of the experiment to evaluate the efficacy of different treatments against mite after first spray are furnished in Table 5. The mean mite counts before the application of treatments ranged from 18.05 to 20.80 per cm^2 leaf area.

One day after spraying, the lowest mean mite count of 4.97/cm^2 leaf area was recorded in the treatment, spiromesifen which was on par with diafenthiuron (5.08/cm^2 leaf area). The botanicals, neem oil and azadirachtin had an average of 8.29 and 8.78 mites per cm^2 leaf area and were on par with each other. The acaropathogen, *A. zeylanicum* applied at 1×10^8 spores ml^{-1} registered a lower mite count of 10.81 as against 15.30 per cm^2 leaf area at 1×10^7 spores ml^{-1}.

Three days after spraying, the plants treated with synthetic acaricide molecules harboured significantly lower mite populations compared to other treatments. Spiromesifen recorded the lowest population of 1.38 mites per cm^2 leaf area which was on par with diafenthiuron (1.97/cm^2 leaf area). This was followed by neem oil (3.46/cm^2 leaf area), azadirachtin (4.15/cm^2 leaf area), *A. zeylanicum* at 1×10^8 spores ml^{-1} (8.70/cm^2 leaf area) and *A. zeylanicum* at 1×10^7 spores ml^{-1} (10.67/cm^2 leaf area). All these treatments were significantly superior to untreated control which recorded a mean population of 29.33 mites per cm^2 leaf area.

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At seven days after spraying, the lowest count of 0.92 mites per cm^2 leaf area was recorded by spiromesifen. This was followed by diafenthiuron (1.07/cm^2 leaf area), neem oil (2.05/cm^2 leaf area) and azadirachtin (2.95/cm^2 leaf area) which were on par with each other. *A. zeylanicum* at 1×10^8 spores ml^{-1} as well as 1×10^7spores ml^{-1} recorded mite count of 5.08 and 8.85 per cm^2 leaf area respectively which significantly differed from untreated control (34.77 mites per cm^2 leaf area).

Seven days after treatment, spiromesifen recorded the highest per cent reduction in the mite count (95.02%) closely followed by diafenthiuron (94.14%). The next best treatment was neem oil which recorded 88.64 per cent reduction in mite count followed by azadirachtin (84.20%). *A. zeylanicum* at both 1×10^8 and 1×10^7 spores ml^{-1} also significantly reduced mean mite count to 72.71 and 55.03 per cent respectively.

At ten days after spraying, spiromesifen recorded the lowest count of 0.68 mites per cm^2 leaf area followed by diafenthiuron (0.85/cm^2 leaf area) and were on par with each other. Neem oil recorded a mite count of 2.38 mites per cm^2 leaf area and was on par with diafenthiuron. The mite count recorded in the treatment azadirachtin (3.62 mites per cm^2 leaf area) was on par with that of neem oil. This was followed by *A. zeylanicum* at 1×10^8 spores ml^{-1} (7.35/cm^2 leaf area) and at 1×10^7 spores ml^{-1} (11.25/cm^2 leaf area) and were superior to untreated control (30.03/cm^2 leaf area).

A similar trend was observed at fourteen days after spraying, where the novel acaricides continued to record lower mite populations. The lowest mite count was recorded by spiromesifen (0.46/cm^2 leaf area) which was on par with diafenthiuron (0.57/cm^2 leaf area). This was followed by botanicals, neem oil (3.33/cm^2 leaf area) and azadirachtin (4.03/cm^2 leaf area), which were on par with each other. *A. zeylanicum* at 1×10^8 spores ml^{-1} recorded a significantly lower mite count (7.14 per cm^2 leaf area) compared to at 1×10^7 spores ml^{-1} (9.09/cm^2 leaf area). The control treatment recorded significantly higher mite count of 30.03 mites per cm^2 leaf area.

Fourteen days after the treatment application, spiromesifen reduced the mite count to the tune of 97.51 per cent, closely followed by diafenthiuron (96.88%). The next best treatment was neem oil which recorded 81.55 per cent reduction in mite count followed by azadirachtin (78.42%). The acaropathogen *A. zeylanicum* at 1×10^{8} spores ml^{-1} reduced mite count to the level of 61.65 per cent followed by 1×10^{7} spores ml^{-1} (53.81%).

Table 5. Effect of various treatments on *Tetranychus truncatus* on cucumber in polyhouse in first experiment

Sl. No.	Treatments		Mean no. of mite/cm² leaf area			Per cent reduction in mite count after 7 days	Mean no. of mite/cm² leaf area		Per cent reduction in mite count after 14 days
		Precount	1 DAS	3 DAS	7 DAS		10 DAS	14 DAS	
1	*Acremonium zeylanicum* 1×10^7 spores ml^{-1}	19.68	15.30 ^{b} (15.31)	10.67^{b} (10.69)	8.85 ^{b} (8.80)	55.03	11.25^{b} (11.26)	9.09 ^{b} (9.13)	53.81
2	*Acremonium zeylanicum* 1×10^8 spores ml^{-1}	18.62	10.81^{c} (10.80)	8.70 ^{c} (8.69)	5.08 ^{c} (5.10)	72.71	7.35 ^{c} (7.34)	7.14 ^{c} (7.12)	61.65
3	Spiromesifen 100g ai ha^{-1}	18.50	4.97 ^{e} (4.95)	1.38 ^{f} (1.36)	0.92 ^{e} (0.94)	95.02	0.68 ^{f} (0.66)	0.46 ^{e} (0.43)	97.51
4	Diafenthiuron 400g ai ha^{-1}	18.29	5.08 ^{e} (5.06)	1.97 ^{f} (1.94)	1.07 ^{e} (1.10)	94.14	0.85 ^{ef} (0.84)	0.57 ^{e} (0.52)	96.88
5	Neem oil 2 %	18.05	8.29 ^{d} (8.27)	3.46 ^{e} (3.42)	2.05 ^{de} (2.09)	88.64	2.38 ^{de} (2.36)	3.33 ^{d} (3.27)	81.55
6	Azadirachtin 0.005 %	18.68	8.78 ^{d} (8.77)	4.15 ^{d} (4.14)	2.95 ^{d} (2.96)	84.20	3.62 ^{d} (3.61)	4.03 ^{d} (4.01)	78.42
7	Control	20.80	22.39 ^{a} (22.42)	29.33 ^{a} (29.39)	34.77^{a} (34.67)	-	30.03 ^{a} (30.06)	30.03^{a} (30.14)	-

DAS = Days after spraying. Means followed by same letters do not differ significantly by DMRT (p = 0.05), Values in the paranthesis are adjusted treatment means.

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4.1.2.1 Efficacy of treatments against *Tetranychus truncatus* on cucumber (Second experiment)

The mean count of mites before imposing second spray application of different treatments ranged from 17.16 to 19.29 per cm^2 leaf area. The results of the field experiment to evaluate the efficacy of different treatments against mite in the second experiment are presented in Table 6.

At first day after spraying, the acaricide molecule, spiromesifen recorded the lowest mean mite count of 3.73 per cm^2 leaf area and was on par with diafenthiuron (4.65/ cm^2 leaf area). The botanicals, neem oil and azadirachtin recorded 7.87 and 8.62 mites per cm^2 leaf area respectively and were on par with each other. Acaropathogen, *A. zeylanicum* at 1×10^8 spores ml^{-1} and at 1×10^7 spores ml^{-1} recorded mean mite counts of 10.10 and 13.41 per cm^2 leaf area respectively. All the treatments were superior to untreated control (20.66/ cm^2 leaf area).

At three day after spraying, the lowest mite count of 1.94 per cm^2 leaf area was recorded by spiromesifen followed by diafenthiuron (2.12/ cm^2 leaf area), and were on par with each other. Neem oil recorded a mite count of 3.17 per cm^2 leaf area and was on par with acaricides, spiromesifen and diafenthiuron. Azadirachtin recorded a mite count of 4.27 per cm^2 leaf area and was statistically on par with neem oil. This was followed by *A. zeylanicum* at 1×10^8 spores ml^{-1} (7.82/cm^2 leaf area) and 1×10^7 spores ml^{-1} (10.46/cm^2 leaf area) and was superior over untreated control (27.6/cm^2 leaf area).

At seven days after spraying also, the treatments recorded similar trend and were superior over untreated control. The lowest mite count of 1.36 per cm^2 leaf area was recorded by spiromesifen which was on par with diafenthiuron (1.5/ cm^2 leaf area). Among the botanicals, neem oil recorded lower mite population of 2.21 per cm^2 leaf area compared to azadirachtin (3.27/cm^2 leaf area) but were on par with each other. The mite count in neem oil was on par with the acaricide molecules. *A. zeylanicum* recorded mite counts of 4.73 and 7.71 mites per cm^2 leaf area at concentrations, 1×10^8 spores ml^{-1} and 1×10^7 spores ml^{-1} respectively.

Seven days after treatment, spiromesifen recorded significant reduction in mite count (92.20%), closely followed by diafenthiuron (91.25%). The next best treatment was neem oil with a mean reduction in mite count of 87.91 per cent followed by azadirachtin (81.88%). The acaropathogen *A. zeylanicum* at 1×10^8 spores ml^{-1} reduced mite count to the level of 74.51 per cent followed by 1×10^7spores ml^{-1} (58.98%).

Similar trend was observed at ten days after spraying where the chemical molecules continued to record lower mite count with spiromesifen recording the lowest mite population of 0.85 per cm^2 leaf area. It was found on par with diafenthiuron (0.93/cm^2 leaf area). A mean mite population of 2.70 per cm^2 leaf area was recorded by neem oil which was superior over azadirachtin (3.73/cm^2 leaf area). *A. zeylanicum* at 1×10^8 spores ml^{-1} recorded a mean mite count of 5.42 as against 9.63 per cm^2 leaf area at the concentration 1×10^7spores ml^{-1} and were superior over untreated control (27.33/cm^2 leaf area).

At fourteen days after spraying, the lowest mean mite population was recorded by spiromesifen (0.54/cm^2 leaf area) which was on par with diafenthiuron (0.60/cm^2 leaf area). Among botanicals, neem oil (3.24/cm^2 leaf area) proved to be superior over azadirachtin (4.33/cm^2 leaf area). This was followed by *A. zeylanicum* at 1×10^8 spores ml^{-1} (6.34/cm^2 leaf area) and *A. zeylanicum* at 1×10^7 spores ml^{-1} (8.37/cm^2 leaf area) and were superior over untreated control (31.19/cm^2 leaf area).

Fourteen days after treatment application, spiromesifen recorded 96.90 per cent reduction in the mite count closely followed by diafenthiuron (96.50%). The next best treatment was neem oil with a mean per cent reduction of 82.27 over precount followed by azadirachtin (76.01%). The acaropathogen *A. zeylanicum* at 1×10^8 spores ml^{-1} reduced mite count to the level of 65.84 per cent followed by 1×10^7spores ml^{-1} (55.47%).

Table 6. Effect of various treatments on *Tetranychus truncatus* on cucumber in polyhouse in second experiment

Sl. No.	Treatments		Mean no. of mites/cm² leaf area			Per cent reduction in mite count after 7 days	Mean no. of mites/cm² leaf area		Per cent reduction in mite count after 14 days
		Precount	1 DAS	3 DAS	7 DAS		10 DAS	14 DAS	
1	*Acremonium zeylanicum* 1×10^7 spores ml^{-1}	18.80	13.41[b] (13.69)	10.46[b] (10.72)	7.71[b] (7.79)	58.98	9.63[b] (9.53)	8.37[b] (8.41)	55.47
2	*Acremonium zeylanicum* 1×10^8 spores ml^{-1}	18.56	10.10[c] (10.26)	7.82[c] (7.98)	4.73[c] (4.77)	74.51	5.42[c] (5.37)	6.34[c] (6.36)	65.84
3	Spiromesifen 100g ai ha^{-1}	17.45	3.73[e] (3.34)	1.94[e] (1.57)	1.36[e] (1.25)	92.20	0.85[f] (0.97)	0.54[f] (0.47)	96.90
4	Diafenthiuron 400g ai ha^{-1}	17.16	4.65[e] (4.11)	2.12[e] (1.61)	1.5[e] (1.35)	91.25	0.93[f] (1.104)	0.60[f] (0.50)	96.50
5	Neem oil 2 %	18.28	7.87[d] (7.89)	3.17[de] (3.19)	2.21[de] (2.21)	87.91	2.70[e] (2.692)	3.24[e] (3.24)	82.27
6	Azadirachtin 0.005 %	18.05	8.62[d] (8.53)	4.27[d] (4.18)	3.27[d] (3.24)	81.88	3.73[d] (3.76)	4.33[d] (4.31)	76.01
7	Control	19.29	20.66[a] (21.19)	27.6[a] (28.10)	30.84[a] (30.98)		27.33[a] (27.16)	31.19[a] (31.28)	

DAS = Days after spraying. Means followed by same letters do not differ significantly by DMRT (p = 0.05), Values in the paranthesis are adjusted treatment means.

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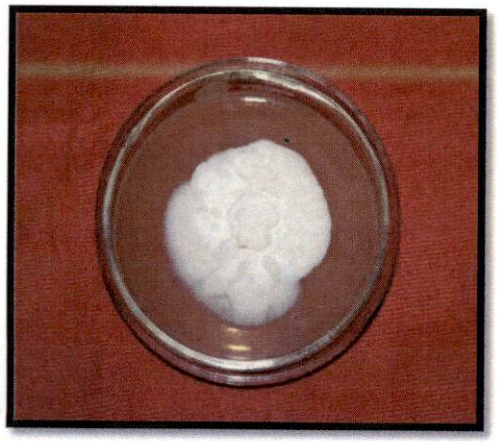
4.2. Assessment of sensitivity of *A. zeylanicum* to different agrochemicals

The effect of different agrochemicals like acaricides, botanicals and fungicides on the growth of *A. zeylanicum* was evaluated using poisoned food technique (Plate 14). The inhibition of fungal growth ranged from 28.15 to 81.48 per cent in the experiment (Table 7).

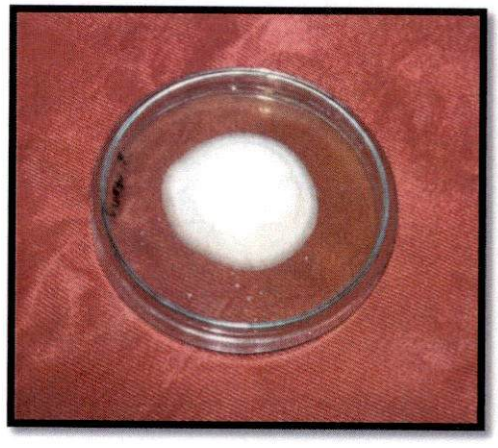
The investigation revealed that fungicides exhibited the highest degree of inhibition of *A. zeylanicum*. The fungicide, Cymoxanil 8% + Mancozeb 64% (Curzate) registered 81.48 per cent inhibition, followed by Famoxadone 16.6%+ Cymoxanil 22.1% (Equation Pro) 80 per cent inhibition. Among the five new acaricide molecules evaluated, fenazaquin recorded the highest growth inhibition of 78.89 per cent and was found to be highly detrimental to the fungus. This was followed by diafenthiuron (72.96%) and propargite (72.22%). The acaricide, fenpyroximate recorded significantly lower growth inhibition (38.52%) than the above acaricides. However, spiromesifen was found to be most compatible with the *A. zeylanicum*, recording the lowest per cent growth inhibition of 28.15 per cent. Azadirachtin was found to be more detrimental among the botanicals which inhibited the growth to an extent of 69.26 per cent as compared to neem oil (62.96%).



Spiromesifen

Diafenthiuron

Fenazaquin



Fenpyroximate

Propargite

Plate 14. Mycelial growth of *Acremonium zeylanicum* in poisoned SDAY media with different agrochemicals

Neem oil

Azadirachtin

Curzate M8

Equation Pro

Control

Plate 14. Mycelial growth of *Acremonium zeylanicum* in poisoned SDAY media with different agrochemicals

Table 7. *In vitro* evaluation of agrochemicals / botanicals against *Acremonium zeylanicum*

Sl. No.	Treatment No.	Treatment	Dose	Radial growth (mm)	Per cent inhibition over control (%)
1	T1	Spiromesifen	0.7 ml l^{-1}	64.67 ^{b} (8.04)	28.15^{e} (5.30)
2	T2	Diafenthiuron	1.6 g l^{-1}	24.33^{e} (4.92)	72.96^{b} (8.53)
3	T3	Fenazaquin	2.5 ml l^{-1}	19.00^{f} (4.35)	78.89^{a} (8.88)
4	T4	Fenpyroximate	1 ml l^{-1}	55.33^{c} (7.43)	38.52^{d} (6.20)
5	T5	Propargite	1.5 ml l^{-1}	25.00^{e} (4.99)	72.22^{b} (8.49)
6	T6	Neem oil	20 ml l^{-1}	33.33^{d} (5.76)	62.96^{c} (7.99)
7	T7	Azadirachtin	5 ml l^{-1}	27.67^{e} (5.25)	69.26^{b} (8.32)
8	T8	Cymoxanil 8% + Mancozeb 64% (Curzate)	2 g l^{-1}	16.67^{f} (4.07)	81.48^{a} (9.02)
9	T9	Famoxadone 16.6%+Cymoxanil 22.1% (Equation pro)	1 ml l^{-1}	18.00^{f} (4.23)	80.00^{a} (8.94)
10	T10	Control	-	90.00^{a} (9.487)	-

Means followed by same letter in the column do not differ significantly by DMRT (P =.05); figures in parentheses are square root transformed values

4.3 Evaluation of safety of *A. zeylanicum* to the predatory mite, *Neoseiulus longispinosus*

4.3.1 Laboratory bioassay

4.3.1.1 Ovicidal effect

The safety of the acaropathogen, *A. zeylanicum* to the eggs of the predatory mite *N. longispinosus* was evaluated under laboratory conditions. The effect of two different concentrations of the fungus on the hatchability and survival rate of the predatory egg is shown in Table 8.

At 24 h, the highest egg hatchability of 76.66 per cent was observed in the untreated control, which was on par with *A. zeylanicum* applied at 1×10^7 spores ml^{-1} with 60 per cent hatchability. The above treatments were followed by 1×10^8 spores ml^{-1} which recorded 50 per cent egg hatchability. The egg hatchability was found to have increased during the period from 24 to 48 h in all the treatments. At 48 h, 100 per cent egg hatchability was recorded in the untreated control. The treatments, 1×10^7 spores ml^{-1} and 1×10^8 spores ml^{-1} recorded 80 and 70 per cent hatchability respectively and were on par with each other. At 72 h, all the eggs tested in the study hatched irrespective of the treatments.

Table 8. Effect of *Acremonium zeylanicum* on the eggs of *Neoseiulus longispinosus*

Sl. No.	Treatment No.	Treatment	Egg hatchability (%)		
			24 h	48 h	72 h
1	T1	1×10^{7} spores ml^{-1}	60.00^{ab} (51.12)	80.00^{b} (66.022)	100.00 (88.83)
2	T2	1×10^{8} spores ml^{-1}	50.00^{b} (45.00)	70.00^{b} (57.256)	100.00 (88.83)
3	T3	Control	76.66^{a} (61.437)	100.00^{a} (88.83)	100.0 (88.83)
CD value (p=0.05)			12.96	13.11	NS

Means followed by same letter in the column do not differ significantly by DMRT (P =.05); Figures in parentheses are arcsine transformed values

4.3.1.2 Adulticidal effect on gravid females of *N. longispinosus*

The investigation on the effect of *A. zeylanicum* on adults of predatory mite, *N. longispinosus* was carried out as a laboratory bioassay and the results of the study are presented in Table 9.

None of the treatments recorded any mortality of the predatory mite one day after treatment. Two days after treatment, 6.67 and 3.33 per cent of predatory mites were killed in the treatments 1×10^{8} spores ml^{-1} and 1×10^{7} spores ml^{-1} respectively, both being par with each other and significantly superior to control. On third day, 1×10^{8} spores ml^{-1} recorded 13.33 per cent mortality which was significantly superior to other treatments. However, four days after treatment, both the spore concentrations, namely, 1×10^{8} spores ml^{-1} and 1×10^{7} spores ml^{-1} recorded 13.33 per cent mortality each. The treatment 1×10^{8} spores ml^{-1} recorded a mortality of 16.67 per cent on both fifth and sixth days of spraying, while 1×10^{7}spores ml^{-1} recorded 13.33 per cent mortality for the corresponding period. The two treatments were on par with each other. At seven days after spraying, the highest mortality of 20 per cent was recorded in the treatment 1×10^{8} spores ml^{-1} and was on par with the treatment 1×10^{7}spores ml^{-1} with 16.67 per cent mortality. Both the treatments induced significantly higher mortality to *N. longispinosus* compared to control, which did not cause any mortality throughout the study period.

Table 9. Effect of *Acremonium zeylanicum* on gravid females of *Neoseiulus longispinosus*

Sl. No.	Treatment No.	Treatment	Per cent mortality						
			1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
1	T1	1×10^{7} spores ml^{-1}	0.00 (1.17)	3.33 ^{a} (1.17)	6.67 ^{ab} (10.34)	13.33 ^{a} (19.51)	13.33 ^{a} (19.51)	13.33 ^{a} (19.51)	16.67 ^{a} (24.09)
2	T2	1×10^{8} spores ml^{-1}	0.00 (1.17)	6.67 ^{a} (10.34)	13.33 ^{a} (19.51)	13.33 ^{a} (19.51)	16.67 ^{a} (24.09)	16.67 ^{a} (24.09)	20.00 ^{a} (26.33)
3	T3	Control	0.00 (1.17)	0.00 ^{b} (1.17)	0.00 ^{b} (1.17)	0.00 ^{b} (1.17)	0.00 ^{b} (1.17)	0.00 ^{b} (1.17)	0.00 ^{b} (1.17)
CD value (p=0.05)			NS	NS	12.90	11.53	8.15	8.15	3.97

Means followed by same letter in the column do not differ significantly by DMRT (P=.05)

Figures in parentheses are arcsine transformed values

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Discussion

5. DISCUSSION

The observations and inferences on the investigations undertaken to evaluate the biocontrol potential of *Acremonium zeylanicum* (Petch) Gams and Evans against the spider mite, *Tetranychus truncatus* Ehara, to assess the sensitivity of the acaropathogen to selected novel acaricides and botanicals and also to evaluate safety of *A. zeylanicum* to the predatory mite, *Neoseiulus longispinosus* (Evans) are discussed below in the light of available literature.

5.1. Evaluation of the biocontrol potential of *A. zeylanicum* against the spider mite *T. truncatus*

The acaropathogen, *A. zeylanicum* could cause more than 50 per cent mortality of adult mite within six days in the laboratory when applied at concentrations of 1×10^8 spores ml^{-1} and 1×10^9 spores ml^{-1} (Fig 1). Both the treatments recorded significantly higher mortality of 68.36 and 61.65 per cent respectively on the seventh day of treatment application. In the polyhouse also, *A. zeylanicum* significantly reduced mite population on cucumber seven days after treatment at both the concentrations of 1×10^8 spores ml^{-1} and 1×10^7 spores ml^{-1}. The study clearly indicated the potential of *A. zeylanicum* in bringing down the population of the spider mite, *T. truncatus*. Pathogenicity studies conducted earlier with several acaropathogens have brought out the regulatory potential of the local isolates of pathogenic fungi. For instance, an entomopathogen *Cladosporium cladosporioides* isolated from *T. urticae* on okra at Coimbatore recorded 96.5 per cent mortality of mites when tested in the laboratory (Jeyarani *et al.*, 2011). Similarly, local strain of *Hirsutella thompsoni,* when evaluated against *Oligonychus coffeae* in tea registered a mortality of 65 per cent in laboratory (Amarasena *et al.*, 2011).

The pathogenicity of *A. zeylanicum* against eggs of *T. truncatus* was markedly different from the adults. The fungus could cause only 41.33 per cent mortality on eggs of *T. truncatus* 96 h after treatment, even at the highest spore concentration of 1×10^9 spores ml^{-1}. This clearly indicates that *A. zeylanicum* is more pathogenic to adult mite than to the egg stage. Variability

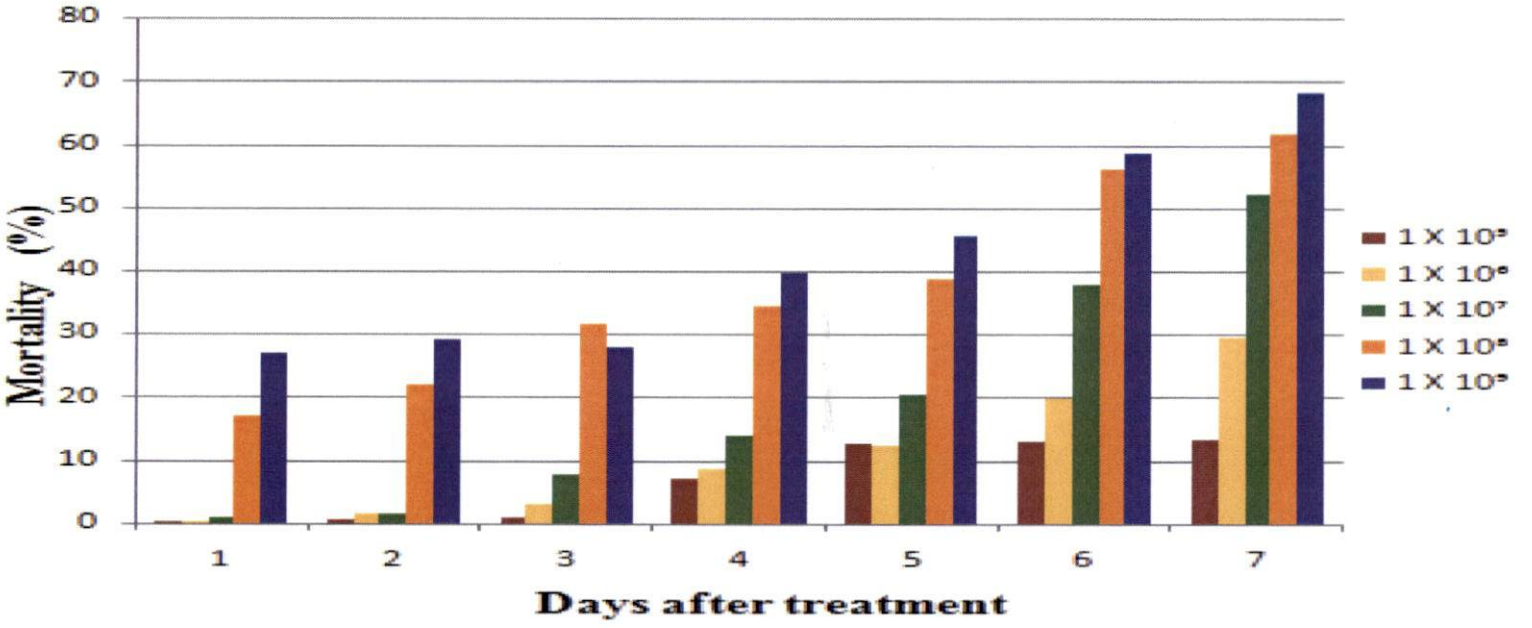

Fig. 1. Adulticidal effect of *Acremonium zeylanicum* at different spore concentrations on *Tetranychus truncatus* in the laboratory

in pathogenicity of different entomopathogenic fungi to different stages of various phytophagous mites has been reported by several workers earlier. Laboratory bioassay of the entomopathogenic fungi *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* against the broad mite, *Polyphagotarsonemus latus* revealed that *P. fumosoroseus, B. bassiana* and *M. anisopliae* at 1×10^8 conidia ml^{-1} recorded 90, 81 and 60 per cent mortality of active mites respectively. However, at the same dosage, *M. anisopliae* could cause only 10 per cent infection on the eggs of *P. latus*, while no infection was caused by either *B.bassiana* or *P. fumosoroseus* (Nugroho and Ibrahim, 2004). In the present study, the per cent hatchability of eggs varied at different spore concentrations tested and delay in hatching was observed with increase in spore concentration. Hatch rates of *T. cinnabarinus* eggs were reported to have decreased due to infection by both *B. bassiana* and *P. fumosoroseus*. At higher conidial concentrations, greater reduction in hatch rates was observed (Weibin and Mingguang, 2004).

It was found that efficacy of the acaropathogen against *T. truncatus* increased with increase in concentration of the pathogen both in laboratory and polyhouse. The highest concentration of 1×10^9 spores ml^{-1} tested in the laboratory recorded the highest mortality. In the polyhouse, at the higher concentration of 1×10^8 spores ml^{-1} the acaropathogen could reduce the mite count by 72.71 and 74.51 per cent on seventh day in first and second experiments respectively. At a lower dosage of 1×10^7spores ml^{-1} *A. zeylanicum* brought about a reduction in mite count by 55.03 and 58.98 per cent by seven days in the first and second experiment respectively. A native isolate of *A. zeylanicum* isolated from sugarcane woolly aphid (SWA) from Dharwad, Karnataka, when evaluated for its pathogenicity against SWA in the laboratory, showed increased mortality of aphid with increase in concentration and duration of exposure. Highest mortality of 83 per cent was recorded at 1×10^{10} conidia ml^{-1} (Patil *et al.*, 2011). Shalini *et al.* (2011) reported *A. zeylanicum* to cause mortality of cotton mirid bug (*Creontiades biseratence* Distant) to the tune of 69.05, 78.57 and 86.24 per cent at concentrations of 1×10^7, 1×10^8 and 1×10^9 conidia /ml respectively under laboratory conditions.

A. zeylanicum isolated from sugarcane woolly aphid from northern Karnataka was evaluated for pathogenicity against important sucking pests of different crops in a laboratory experiment at Dharwad. The fungus proved to be highly pathogenic to cabbage aphid (*Brevicoryne brassicae* Linn.), sorghum aphid (*Melanaphis sacchari* Zehnt.) and sugarcane woolly aphid (*Ceratovacuna lanigera* Zehnt.). However, it was relatively less pathogenic to chilli mite (*Polyphagotarsonemus latus* Banks) and brinjal mite (*Tetranychus neocaledonicus* Andre) (Divan and Mallapur, 2011). But in the present study, the fungus *A. zeylanicum* was found to be highly pathogenic to *T. truncatus* recording a highest mortality of 68.36 per cent at a spore concentration of 1×10^9 spores per ml. This might be because the fungal isolate evaluated in the present study was isolated from a mycosed spider mite and not from other hosts and hence highly adaptable to the host and locality. Pena *et al.* (1996) found that fungal isolates originating from *Polyphagotarsonemus latus* Banks (Tarsonomidae) were more pathogenic to this mite species than those isolated from other hosts.

In the present study, though the mite population was significantly declined by seventh day after application of *A. zeylanicum* in the polyhouse, there was an increase in population from seventh day to fourteenth day (Fig 2 & 3). Laboratory bioassay has indicated comparatively poor ovicidal action of *A. zeylanicum* against *T. truncatus* during the study. As a result, it could be that a considerable proportion of eggs in the population would have hatched during this period, leading to increase in population by fourteenth day in the polyhouse.

The study showed that the efficacy of *A. zeylanicum* was not comparable with that of novel acaricides and botanicals. The new acaricide molecules, spiromesifen and diafenthiuron were effective and superior to the fungus in reducing the population of *T. truncatus*. Efficacy of these acaricides in reducing mite population was pronounced from the first day after spray application itself. In the present study spiromesifen recorded 97.51 and 96.70 per cent reduction in mite population after 14 days in first and second experiment respectively. Baloch *et al.* (2016) also reported that spiromesifen resulted in significant reduction of

96.27 per cent in the population of *T. urticae* 15 days after treatment application on okra. Study on the efficacy of spiromesifen against *T. urticae* on ridge gourd showed that the molecule caused more than 90 per cent mortality (Reddy and Latha, 2013). Under field conditions, spiromesifen could result in complete suppression of population of *T. urticae* in ten days time (Sato *et al.*, 2011).

Diafenthiuron also recorded significant reduction in mite population by 96.88 and 96.50 per cent respectively at fourteen days in first and second experiments which was on par with spiromesifen. High efficacy of diafenthiuron in suppressing population of spider mite in different crops was earlier reported by several workers (Patil, 2005; Bhaskaran *et al.*, 2007)

Among the botanicals evaluated neem oil (81.55% and 82.27%) and azadirachtin (78.42% and 76.01%) recorded considerable reduction in mite population in the first and second experiment. This was in accordance with the observation of Krishna and Bhaskar (2016) who reported that neem oil 2 per cent recorded 81.15 per cent reduction in population of *T. urticae* on okra. Kumar (2007) observed that neem oil two per cent was effective in managing mite population on rose cultivated under polyhouse condition. Acaricidal property of azadirachtin was reported by Bernandi *et al.* (2012) who recorded a reduction in the population of *T. urticae* on strawberry by 94 to 100 per cent.

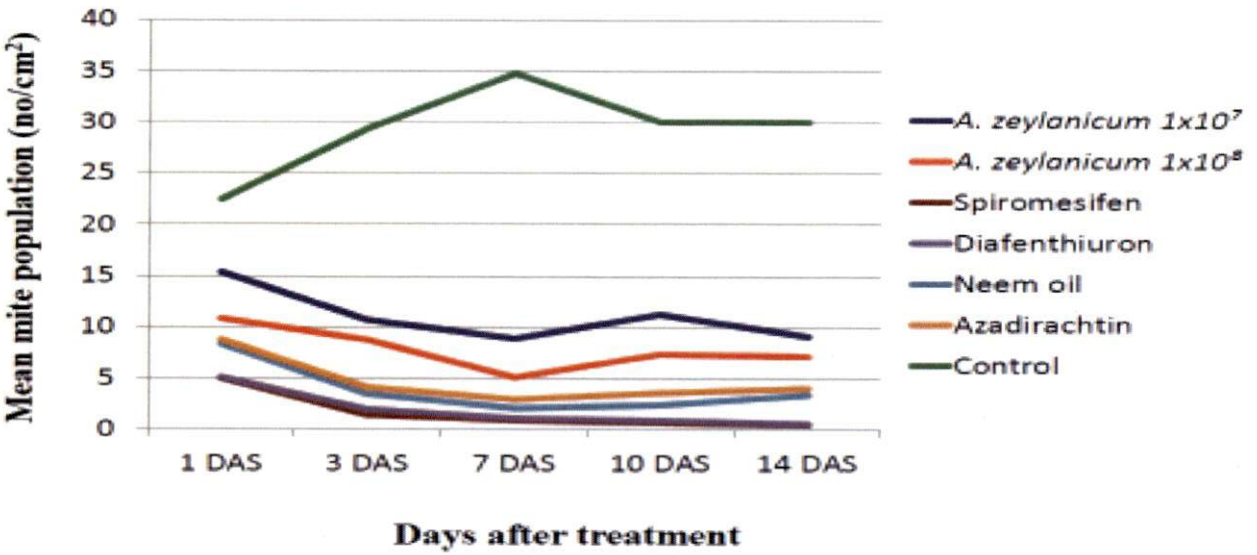

Fig. 2. Efficacy of treatments against *Tetranychus truncatus* in polyhouse (first experiment)

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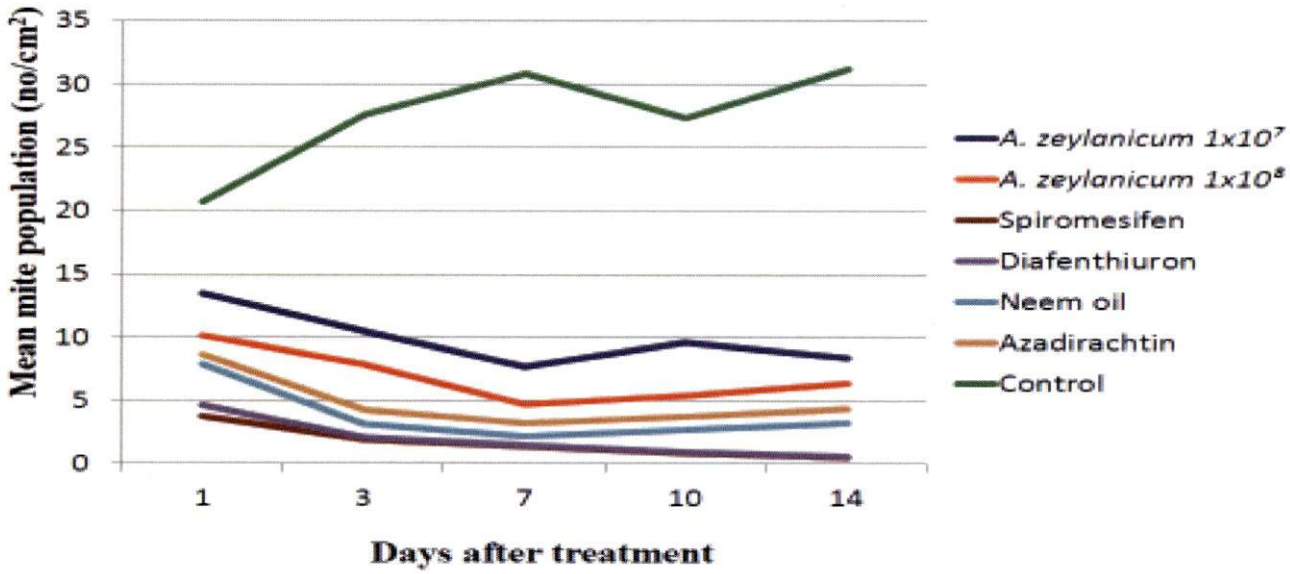

Fig. 3. Efficacy of treatments against *Tetranychus truncatus* in polyhouse (second experiment)

5.2. Assessment of sensitivity of the fungus to different agrochemicals/ botanicals

The acaropathogen *A. zeylanicum* was tested for its compatability with different agrochemicals like acaricides, botanicals and fungicides that are commonly used for the mite and disease management in cucumber. In integrated pest management programmes, the knowledge of compatibility was imperative to minimize deleterious effects on biocontrol agents/ natural enemies and a synergistic combination of microbial agents with other technologies would appreciate the attributes of entomopathogens in near future (Lacey *et al.*, 2001).

The results of the present study revealed that fungicides, Curzate (cymoxanil 8% + mancozeb 64%) and Equation Pro (famoxadone 16.6% + cymoxanil 22.1%) were found to be most detrimental to the acaropathogen *A. zeylanicum* causing highest per cent growth inhibition of 81.48 and 80 per cent respectively (Fig 4). The detrimental effect of commonly used fungicides against entomopathogenic fungi belonging to the class Hyphomycetes was reported earlier by several scientists. Prasanna and Mallapur (2011) observed that the fungicides carbendazim, mancozeb, copper oxychloride and penconazole proved to be highly detrimental to *A. zeylanicum* and recorded 83.35, 79.80, 79.16 and 77.74 per cent inhibition at recommended dose, respectively. The fungicides carbendazim, benomyl and mancozeb significantly or totally inhibited growth of Hyphomycetes entomopathogenic fungi *B. bassiana* and *M. anisopliae* (Faraji *et al.*, 2016). Rachappa *et al.* (2007) reported that carbendazim, propiconazole and chlorothalonil were highly detrimental to *M. anisopliae* with cent per cent growth inhibition.

Compatability studies of acaricides with acaropathogens help to assess the antagonistic or synergistic effect of chemical on the acaropathogenic fungi. In the present investigation, all acaricides recorded different levels of inhibition on growth of *A. zeylanicum*. Among the different acaricides tested in the present study fenazaquin, diafenthiuron and propargite were highly toxic to the fungus with 78.89, 72.96 and 72.22 per cent inhibition respectively. Propargite at 0.1 per cent

and diafenthiuron at 0.08 per cent were also found to be detrimental to the entomopathogenic fungus, *M. anisopliae* with 67.1 and 70.9 per cent mycelial growth inhibition (Dutta *et al.*, 2015). The acaricides spiromesifen and fenpyroximate were found to be relatively safer to the fungus showing 28.15 and 38.52 per cent inhibition respectively. These two molecules were earlier reported to be less detrimental to several entomopathogens (Nissay *et al.* 2012; Waked *et al.* 2015). Cuthbertson *et al.* (2008) reported the safety of spiromesifen to *Lecanicillium muscarium* which recorded 64 per cent spore germination. Oliveira and Neves (2004) evaluated the compatability of *B. bassiana* with fenpyroximate and found the acaricide less detrimental in all the tested concentrations.

Among the botanicals screened, azadirachtin was found to be detrimental to *A. zeylanicum,* recording 69.26 per cent inhibition followed by neem oil with 62.96 per cent inhibition. Neem based products were reported to be incompatible with entomopathogenic fungi in several studies. Azadirachtin was found inhibitory to *Nomurea rileyi* which caused 61.33 per cent radial growth inhibition (Hazarika *et al.*, 2016). There was a significant reduction in the mycelial growth of the *B. bassiana* culture when grown with azadirachtin, showing a strong inhibition of *B. bassiana* at five and ten days after treatment (Hernandez *et al.*, 2012). Neem oil was found to be incompatible with *B. bassiana*, by inhibiting vegetative growth and decreasing production and viability of conidia particularly at higher concentrations (Depieri *et al.*, 2005). Hirose *et al.* (2001) reported negative effects caused by emulsible neem oil to the entomopathogen *B. bassiana* and *M. anisopliae*.

Alves *et al.* (1998), however, pointed out that results obtained *in vitro* should be confirmed under field conditions since environmental factors may also govern the compatibility of entomopathogenic fungi agrochemicals evaluated.

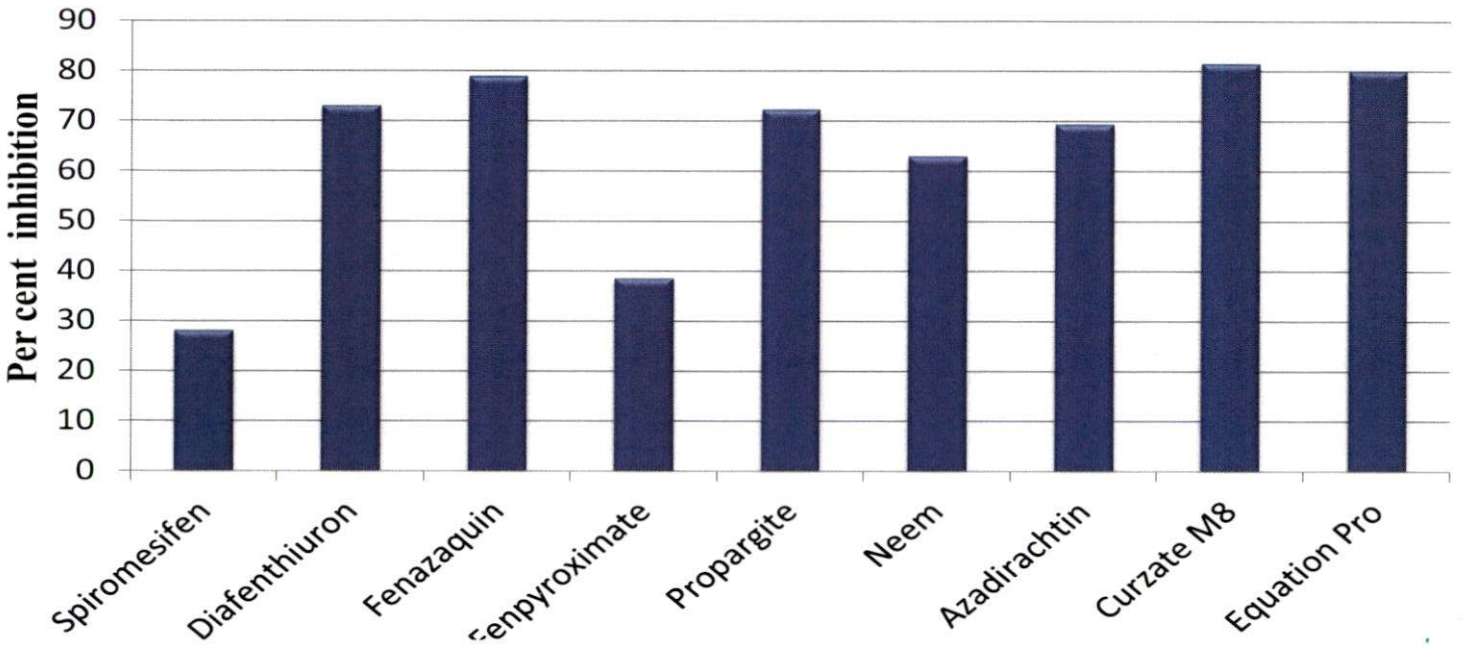

Fig. 4. *In vitro* inhibition of *Acremonium zeylanicum* by different agrochemicals

5.3. Evaluation of safety of *A. zeylanicum* to the predatory mite, *Neoseiulus longispinosus*

Assessment of compatability of synthetic chemicals and biocontrol agents for mite management has always been an interesting area for developing an integrated mite management programme. The phytoseiid mite, *N. longispinosus* has been identified as a potential predator of spider mites both under protected cultivation and open fields (Mallik *et al.* 1998; Onkarappa, 1999). For exploiting *N. longispinosus* as a major component of integrated mite management programme, studies on compatibility of the mite species with different components of IPM is necessary.

Laboratory evaluation of safety of *A. zeylanicum* to the eggs and adults of *N. longispinosus* revealed that the acaropathogen could cause significant mortality against *T. truncatus*, but proved less detrimental to *N. longispinosus* (Fig 5 & 6). *Neosieulus longispinosus* is a predatory mite belonging to the family Phytoseiidae. Phytoseiid mites are characterized by sclerotized body with distinct sclerotized dorsal and ventral shields unlike spider mites belonging to the family Tetranychidae (Krantz and Walter, 2009). Hence the pathogen might have failed to penetrate the cuticle of the predatory mite as easily compared to *T. truncatus,* resulting in low level of infection. According to Wu *et al.* (2014) when the predatory mite, *Neosieulus barkeri* was treated with *B. bassiana* strain SZ-26, the conidia could adhere to the cuticle of adults and germinate, but were not observed to penetrate the cuticle. Conidia were shed gradually from the body, leaving the secretions on the surface of the cuticle, thus causing no pathogenicity to *N. bakeri.*

It was also reported that phytoseiid mites are capable of removing spores of fungal pathogen from their body thus avoiding infection. Wekesa *et al.* (2007) reported the self-grooming behavior in predatory mite, *Phytoseiulus longipes* and the predator was efficient in removing most capilliconidia of the fungal pathogen, *Neozygites floridana.* The infection by entomopathogenic fungi is the result of mechanical pressure on cuticle along with and production of proteinases which are associated with cuticle degradation (Leger *et al.*, 1986).Thus the acaropathogenic

fungi *A. zeylanicum* evaluated in the present study was found to be safer to the predatory mite, *N. longispinosus*.

The results of the present study clearly indicate that *A. zeylanicum* has potential in suppressing mite population. It was found to be compatible, with the commonly used novel acaricicdes namely spiromesifen and fenpyroximate. It was also found to be less detrimental to the predatory mite, *N. longispinosus* which is the major predatory mite encountered in vegetable field. Based on the results of the present study, it is suggested that the local isolate of *A. zeylanicum* is an ideal candidate for incorporation in integrated mite management programme in crops.

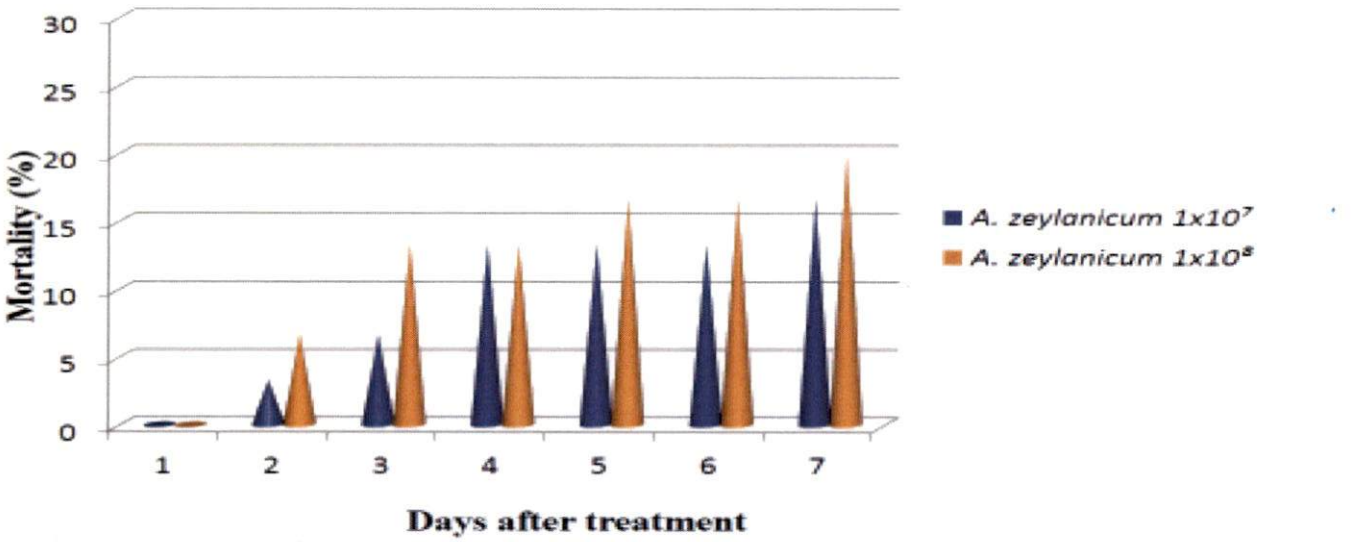

Fig. 5. Adulticidal effect of *Acremonium zeylanicum* at two different concentrations on *Neoseiulus longispinosus*

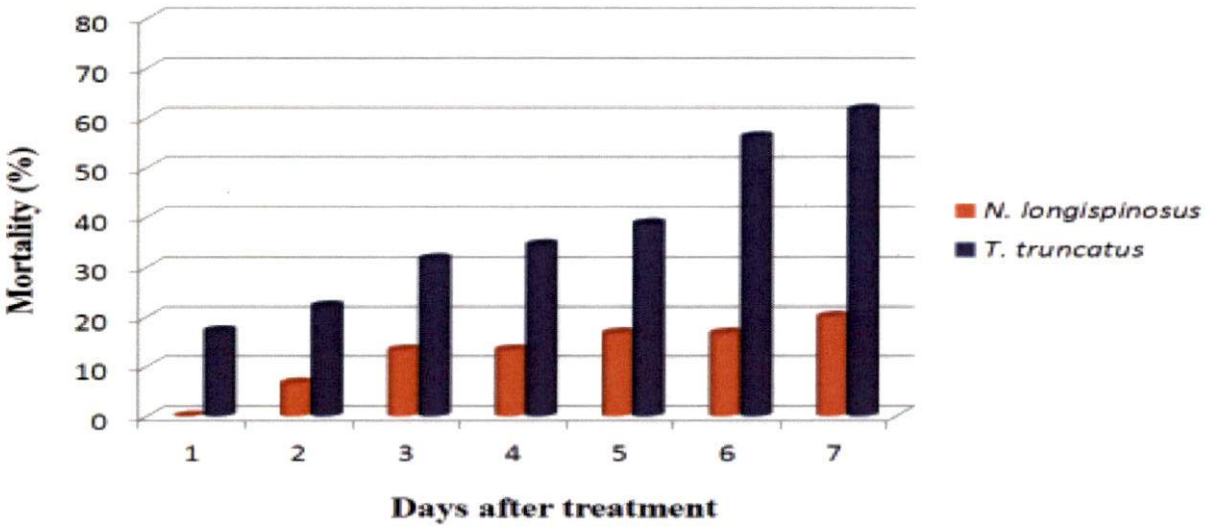

Fig. 6. Effect of *Acremonium zeylanicum* at 1x10^{8} spores ml^{-1} on prey, *Tetranychus truncatus* and predator, *Neoseiulus longispinosus*

Summary

6. SUMMARY

The present study entitled " Bioefficacy of the acaropathogen, *Acremonium zeylanicum* (Petch) Gams and Evans against the spider mite, *Tetranychus truncatus* Ehara (Acari: Tetranychidae)" was carried out at the Acarology laboratory, Department of Agricultural Entomology, College of Horticulture, Vellanikkara during March 2016 – May 2017 to evaluate the biocontrol potential of *A. zeylanicum* against the spider mite, *T. truncatus*, to assess the sensitivity of the acaropathogen to selected novel acaricides and botanicals and also to evaluate safety of *A. zeylanicum* to the predatory mite, *Neoseiulus longispinosus* (Evans).

The salient findings of the study are summarized hereunder

- The laboratory bioassay conducted to evaluate the efficacy of the fungal isolate, *A. zeylanicum* at five different concentrations namely 1×10^5 spores ml^{-1}, 1×10^6 spores ml^{-1}, 1×10^7 spores ml^{-1}, 1×10^8 spores ml^{-1} and 1×10^9 spores ml^{-1} along with an untreated control against *T. truncatus* indicated that *A. zeylanicum* was more effective against adult mites compared to egg stage. At the highest concentration (1×10^9 spores ml^{-1}), the fungus recorded maximum egg mortality of 41.33 per cent at 96 h of treatment.
- The fungus proved to be significantly more pathogenic to adult mites at 7 days after spraying. More than 50 per cent mortality of mites were recorded within six days of spraying in treatments 1×10^9 spores ml^{-1} and 1×10^8spores ml^{-1}. The mortality of *T. truncatus* increased with increase in concentration of the fungus. On the seventh day, 1×10^9 spores ml^{-1} recorded the highest mortality of 68.36 per cent followed by the treatments, 1×10^8 spores ml^{-1} (61.65%), 1×10^7 spores ml^{-1} (52.00%), 1×10^6 spores ml^{-1} (29.49%) and 1×10^5 spores ml^{-1} (12.28%).
- A field study was conducted to evaluate the efficacy of the acaropathogenic fungi, *A. zeylanicum* at two different concentrations along with two newer acaricide molecules *viz*., spiromesifen and diafenthiuron and two botanicals *viz*., neem oil (2%) and azadirachtin (0.005%) and an untreated control

against *T. truncatus* on cucumber during March 2017 to May 2017 in two experiments. The local isolate of *A. zeylanicum* significantly reduced mite population seven days after treatment at both the concentration of 1×10^8 spores ml^{-1} and 1×10^7 spores ml^{-1}.

- At the higher concentration of 1×10^8 spores ml^{-1} the acaropathogen reduced the mite count by 72.71 and 74.51 per cent in first and second experiments respectively. Similarly, at a lower concentrations of 1×10^7 spores ml^{-1} *A. zeylanicum* brought about a reduction in mite count by 55.03 and 58.98 per cent in first and second experiment respectively.
- The study showed that the efficacy of *A. zeylanicum* was not comparable with that of novel acaricides and botanicals. The new acaricides spiromesifen and diafenthiuron recorded high efficacy against *T. truncatus* and observed significant reduction in mite population. Among the botanicals evaluated, neem oil (2%) was found to perform better than azadirachtin though they were on par with each other.
- Assessment of sensitivity of *A. zeylanicum* to different agrochemicals revealed that the acaropathogen was highly sensitive to the fungicides Curzate (cymoxanil 8% + mancozeb 64%) and Equation Pro (famoxadone 16.6%+ cymoxanil 22.1%) recording highest per cent inhibition. This was followed by acaricides fenazaquin, diafenthiuron and propargite. The botanicals azadirachtin and neem oil were also found detrimental to *A. zeylanicum*. However, *A. zeylanicum* was less sensitive to acaricides spiromesifen and fenpyroximate.
- Laboratory studies on the safety of *A. zeylanicum* on the predatory mite *N. longispinosus* revealed that, the fungus was found less detrimental to the predatory mite compared to the prey mite, *T. truncatus*. *A. zeylanicum* did not show any effect on the eggs of the predatory mite. However, a maximum of 20 per cent mortality was recorded on active stages of *N. longispinosus* when *A. zeylanicum* was applied at the concentration 1×10^8 spores ml^{-1}.

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Appendix

APPENDIX 1

The composition of Sabouraud Dextrose Agar (SDAY) medium is given below

1. Peptone : 10 g
2. Dextrose : 40 g
3. Yeast extract : 20 g
4. Agar : 20 g
5. Distilled water : 1000 ml

BIOEFFICACY OF THE ACAROPATHOGEN, *Acremonium zeylanicum* (PETCH) GAMS AND EVANS AGAINST THE SPIDER MITE, *Tetranychus truncatus* EHARA (ACARI: TETRANYCHIDAE)

by

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(2015-11-010)

ABSTRACT OF THE THESIS
Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture
(AGRICULTURAL ENTOMOLOGY)
Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE
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2017

Abstract

Spider mites (Prostigmata: Tetranychidae) cause severe injury to vegetables grown under both protected and open field conditions. Awareness on the ill effects associated with the use of synthetic chemicals in pest management has resulted in an increased demand for safe to eat food. Hence, ecofriendly strategies for the management of mite assume high priority. Investigations on potential natural enemies of spider mites by All India Network Project on Agricultural Acarology (AINPAA) have identified an acaropathogenic fungus, *Acremonium zeylanicum* (Petch) Gams and Evans from the spider mite, *Tetranychus urticae* Koch on brinjal from Thrissur district.

The present study was undertaken at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara during 2016 - 2017 in the above context to investigate the biocontrol potential of the acaropathogen, *A. zeylanicum* against the predominant species of spider mite in Kerala, *Tetranychus truncatus* Ehara; to assess the sensitivity of the acaropathogen to selected novel acaricides and botanicals and also to evaluate its safety to the predatory mite, *Neoseiulus longispinosus* (Evans).

Laboratory bioassay conducted to evaluate the efficacy of *A. zeylanicum* at five different concentrations *viz*.1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 spores ml^{-1} against *T. truncatus* recorded more than 50 per cent mortality of adult mites within six days at both 1×10^8 and 1×10^9 spores ml^{-1}. The mortality increased with increase in concentration of the fungus and also with progress in time. The highest mortality of 68.36 per cent was recorded by *A. zeylanicum* applied at the rate of 1×10^9 spores ml^{-1} seven days after treatment, which was on par with 61.65 per cent mortality recorded at 1×10^8 spores ml^{-1}. The fungus was more effective against adult stage compared to egg stage of *T. truncatus*. The highest mortality of egg stage (41.33%) was recorded four days after the treatment at 1×10^9 spores ml^{-1}.

Acremonium zeylanicum was evaluated along with two novel acaricides and two botanicals against *T. truncatus* on cucumber under polyhouse conditions. *A. zeylanicum* significantly reduced mite population seven days after treatment at both 1×10^8 spores ml^{-1} (72.71%) and 1×10^7 spores ml^{-1} (55.03%). However the novel acaricides (spiromesifen & diafenthiuron) and botanicals (neem oil & azadirachtin) were significantly superior to the acaropathogen in reducing the mite population.

Compatibility study of *A. zeylanicum* with different agrochemicals revealed that the acaricides, spiromesifen and fenpyroximate were relatively safer to the fungus. The fungicides, Curzate M8 (Cymoxanil 8% + Mancozeb 64 %) and Equation Pro (Fomaxadone 16.6%+ Cymoxanil 22.1%) recorded highest per cent inhibition and were followed by the acaricides, fenazaquin, diafenthiuron, propargite and the botanicals azadirachtin and neem oil. Laboratory evaluation of safety of *A. zeylanicum* to the predatory mite *N. longispinosus* showed that the predatory mite was less susceptible to *A. zeylanicum* than the prey mite, *T. truncatus*. The pathogen did not have any ovicidal effect and had caused much lower mortality of 20 per cent on adults of *N. longispinosus* at the highest dose of 1×10^8 spores ml^{-1}.

The study indicated the potential of *A. zeylanicum* in reducing mite population significantly both in laboratory and polyhouse. The pathogen was found to be compatible with commonly used acaricides and also was safe to the predominant predatory mite species in vegetable ecosystems. Thus it can be inferred that the acaropathogen *A. zeylanicum* could be a valuable component in integrated mite management programme in vegetables.