BIOEFFICACY OF BOTANICALS AGAINST THE SPIDER MITE, *TETRANYCHUS TRUNCATUS* EHARA (PROSTIGMATA: TETRANYCHIDAE)

by

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(2018-11-070)

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KERALA, INDIA

2020

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

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VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2020

DECLARATION

I, hereby declare that the thesis entitled **"Bioefficacy of botanicals against the spider mite, *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"Bioefficacy of botanicals against the spider mite, *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)"** is a record of research work done independently by **Ms. Laya A. C.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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TABLES OF CONTENTS

CHAPTER	TITLE	PAGE NO.
1.	INTRODUCTION	1-2
2.	REVIEW OF LITERATURE	3-18
3.	MATERIALS AND METHODS	19-30
4.	RESULTS	31-63
5.	DISCUSSION	64-84
6.	SUMMARY	85-87
	REFERENCES	i-xii
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Botanicals screened for acaricidal activity against *Tetranychus truncatus*	20
2	Effect of aqueous extract of botanicals on eggs of *Tetranychus truncatus*	33-35
3	Effect of aqueous extract of different botanicals on adults of *Tetranychus truncatus*	38-40
4	Per cent yield of methanolic extract of botanicals	41
5	Effect of methanol extract of botanicals on eggs of *Tetranychus truncatus*	43-44
6	Effect of methanol extract of botanicals on gravid females of *Tetranychus truncatus*	47-48
7	Per cent yield of solvent fractions of *Acorus calamus*	49
8	Effect of *Acorus calamus* solvent fractions on eggs of *Tetranychus truncatus*	51-52
9	Effect of *Acorus calamus* solvent fractions on adults of *Tetranychus truncatus*	54-55
10	Effect of treatments on *Tetranychus truncatus* on cucumber in polyhouse	58
11	Effect of sequential methanol fraction of *Acorus calamus* on egg of *Neosieulus longispinosus*	60
12	Effect of sequential methanol fraction of *Acorus calamus* on adult of *Neosieulus longispinosus*	62
13	Effect of methanol fraction of *Acorus calamus* on acaropathogen, *Acremonium zeylanicum*	63

LIST OF PLATES

Plate No.	Title	Page No. Between
1	Fresh and dry plant parts a. *Lantana camara* b. *Aegle marmalos* c. *Eucalyptus* sp.	20- 21
2	Fresh and dry plant parts a. *Vitex negundo* b. *Ocimum sanctum* c. *Quassia indica*	20-21
3	Fresh and dry plant parts a. *Bacopa monnieri* b. *Carica papaya* c. . *Annona squamosa*	20-21
4	Dried *Acorus calamus* rhizome	20-21
5	Preparation and storage of botanicals	20-21
6	Mass culture of *Tetranychus truncatus* on mulberry leaves	22-23
7	Preparation of aqueous extracts	22-23
8	Experimental setup for ovicidal bioassay of aqueous extracts	22-23
9	Topical application for ovicidal bioassay	24-25
10	Leaf dip method for adulticidal bioassay	24-25

Plate No.	Title	Page No. Between
11	Solvent evaporation using rotary evaporator	24-25
12	Experimental set up for ovicidal bioassay of methanol extracts	24-25
13	Solvent fractions of *Acorus calamus*	26-27
14	Experimental set up for ovicidal bioassay of solvent fractions of *Acorus calamus*	26-27
15	Experiment in polyhouse	26-27
16	Release of mites on cucumber plant	26-27
17	Spraying of botanical extracts	26-27
18	Recording mite population using window count	26-27
19	Effect of 0.7 % methanol fraction of *Acorus calamus* on eggs of *Tetranychus truncatus*	52-53
20	Effect of 0.7 % methanol fraction of *Acorus calamus* on adults of *Tetranychus truncatus*	52-53
21	Effect of 0.7 % methanol fraction of *Acorus calamus* on eggs of *Neoseiulus longispinosus*	62-63
22	Effect of 0.7 % methanol fraction of *Acorus calamus* on adult of *Neoseiulus longispinosus*	62-63
23	Growth inhibition of *Acremonium zeylanicum* in SDAY media poisoned with methanol fraction of *Acorus calamus*	63-64

LIST OF FIGURES

Figure no.	Title	Page no.
1	Effect of aqueous extract of different botanicals on adults of *Tetranychus truncatus*	66
2	Effect of methanol extract of botanicals on gravid females of *Tetranychus truncatus*	69
3	Effect of *Acorus calamus* solvent fractions on eggs of *Tetranychus truncatus*	73
4	Effect of *Acorus calamus* solvent fractions on adults of *Tetranychus truncatus*	74
5	Effect of treatments on *Tetranychus truncatus* on cucumber in polyhouse	76
6	Effect of methanol fraction of *Acorus calamus* on egg of *Neosieulus longispinosus*	79
7	Effect of methanol fraction of *Acorus calamus* on adults of *Neosieulus longispinosus*	80
8	Effect of methanol fraction of *Acorus calamus* on acaropathogen, *Acremonium zeylanicum*	84

Introduction

1. INTRODUCTION

Spider mites of the family Tetranychidae are serious phytophagous pests associated with various field crops, ornamentals and medicinal plants around the world, with potential to cause significant yield losses (Roy *et al*., 2011). In India, spider mites of the genus *Tetranychus* are serious pests of vegetable crops and are reported to cause an yield loss of 7 to 48 per cent (Srinivasa and Sugeetha, 1999). The mites colonise lower surface of leaf, near the midrib and veins. However, during severe infestation, it can also be observed on the upper surface of leaves and twigs, covered with webbing. Chlorotic and stippled appearance on the leaves is the early symptom of infestation by red spider mites. Heavily infested leaves turn completely pale, dry up, and fall off. Affected leaves show deformation, reduced rate of photosynthesis and lower chlorophyll content.

Among the different species of spider mites, *Tetranychus truncatus* Ehara is the predominant one infesting vegetable crops in Kerala (Bennur *et al*., 2015). Vegetable growers in Kerala, are solely dependent on synthetic acaricides for mite management due to their quick action. However, excessive and indiscriminate use of synthetic acaricides has resulted in development of resistance in mites, in addition to adverse effect on non target organisms and environmental contamination with toxic residues. Recently, field population of *Tetranychus truncatus* in Thrissur district, Kerala is reported to have developed significant resistance to commonly used novel acaricides *viz.*, spiromesifen, fenazaquin and diafenthiuron (Bacchar *et al*., 2019). Hence there is a need to identify effective, viable and safer alternatives to synthetic acaricides for its management.

In view of developing ecofriendly safer strategies for mite management in vegetables, many studies have been undertaken in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara under the All India Network Project on Agricultural Acarology. The studies identified the potential of a local isolate of the acaropathogen, *Acremonium zeylanicum* and a native strain of the predatory mite, *Neoseiulus longispinosus* in reducing the population of *T. truncatus*

under protected cultivation (Sherief and Bhaskar, 2018; Lenin and Bhaskar, 2019). Horticultural mineral oil, a high grade paraffinic oil has shown high efficacy against *T. truncatus*, with potential to replace synthetic acaricides (Yadav *et al*., 2019; Yadav and Bhaskar, 2020).

Exploitation of botanicals has been recognised as an alternative to synthetic pesticides, with least risk to human and environment. Studies on plant products and essential oil derived from plants have led to identification of many biologically active chemicals useful in plant protection, mainly against insect pests, weeds and plant pathogenic fungi and also bacteria (Eldoksch *et al*., 2009). These plant products show rapid degradation into environment-friendly components with reduced pesticide accumulation as compared with the synthetic acaricides (Vasquez *et al*., 2016).

Chemicals extracted from different plants are shown to possess high acaricidal property without causing any pollution to environment, nor adversely affecting non target population (Al-Samarrai *et al.*, 2012). However, botanical extracts have not been evaluated and effectively utilized for mite pest management in Kerala so far.

The study entitled "Bioefficacy of botanicals against the spider mite, *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)" was conducted to identify the acaricidal properties of botanicals for utilization in mite management. The objectives of the study include:

1. To evaluate the efficacy of botanical extracts against *T. truncatus*
2. To identify the bioactive solvent fractions of promising plant extracts against *T. truncatus*
3. To test the safety of bioactive fraction of the promising botanical to the predatory mite, *Neoseiulus longispinosus* and acaropathogen, *Acremonium zeylanicum*

Review of literature

2. REVIEW OF LITERATURE

Recently, plant products are being largely exploited in crop protection strategies against economically important insect pests. Numerous studies have documented the toxicity of various botanical extracts, yet only few among them are commercially available in agricultural sector. Literature pertaining to acaricidal activity of botanical extracts is reviewed in this chapter. Since literature on bioefficacy of botanicals against *Tetranychus truncatus* is scanty, information on other mite species and insect pests are also reviewed here.

2.1. BOTANICAL EXTRACTS IN INSECT AND MITE PEST MANAGEMENT

2.1.1. Bioefficacy of botanicals extracts against insect pests

Aqueous extract of various botanicals were found to have significant influence on the mortality, fecundity and hatchability of some insect pests, which can further be exploited effectively in pest management strategies.

Hewage *et al*. (1997) evaluated different solvent extracts of 94 medicinal plants against the groundnut aphid, *Aphis craccivora* and the diamondback moth, *Plutella xylostella.* After 24 h of treatment, significantly higher morality was recorded in the hexane extract of *Pleiospermium alatum* (90%), methanol extracts of *Costus specious* (90%), *Ocimum gratissimum* (86.66%) and *Celtis cinnamomea* (*Celtis timorensis*) (83.33%) against *A. craccivora*. However against *P. xylostella*, significantly higher mortality was recorded by hexane extract of *P. alatum* (93.33 %) and methanolic extract of *Hortonia anguistifolia* (86.66 %), after 24 h of treatment.

Partially purified flavonoids extracted from leaves of *Annona squamosa* at 0.09 mg/ml concentration caused 99.00 per cent mortality of *Callosobruchus chinensis*, 9 h after treatment (Kotkar *et al*., 2001).

Lee *et al*. (2002) fractionated methanol extract of *Acorus gramineus* using hexane, chloroform, ethyl acetate and water and evaluated its direct contact toxicity at 2500 ppm against *Nilaparvata lugens, Myzus persicae, Plutella xylostella* and *Spodoptera litura* after 48 h. The effect of the active constituents, *cis* and *trans* asarone were also studied against these insects at different concentrations. Cent per cent mortality was recorded in both *N. lugens* and *P. xylostella* when treated with hexane extract (2500 ppm) as well as *cis* and *trans* asarone (2000 ppm).

Leatemia and Isman (2004) investigated the insecticidal activity of crude ethanolic seed extracts of *Annona squamosa, A. muricata, Lansium domesticum* and *Sandoricum koetjape* against *Spodoptera litura. Annona squamosa* showed larval growth ranging from 8.3-66.9 per cent, while *A. muricata* recorded 17.8-96.0 per cent growth. The extracts of *L. domesticum* and *S. koetjape* were found ineffective, recording 78-118 and 49-97 per cent larval growth relative to control, respectively.

Acorus calamus fractions were evaluated for insecticidal activity against the storage pests, *Tribolium confusum* and *Sitophilus oryzae*. Petroleum ether and acetone extract reported 99 per cent mortality of *T. confusum* at 0.157 mg/cm^2 concentration after 72 h, whereas methanol extract was found ineffective. At the same concentration and time interval, petroleum ether, methanol and acetone extracts recorded 99, 92 and 80 per cent mortality of *S. oryzae*. Mean repellency of 98.67 and 69.33 per cent was recorded against *T. castaneum* and *T. confusum*, respectively at 628.76 µg/cm^2 concentration (Hossain *et al*., 2008).

Leaf and rhizome powder of *A. calamus*, as well as the methanol and petroleum ether extracts of the powders were evaluated against the pulse beetle, *Callosobruchus chinensis*. Leaf and rhizome powder coated on chickpea seed at concentration of 20 mg/g seed caused 91.1 and 100 per cent mortality, respectively. The methanolic extract of leaf and rhizome at 0.4 mg/g seed recorded mortality of 100 and 92.4 per cent, respectively, whereas the petroleum ether extract recorded 69.9 and 92.2 per cent mortality, respectively (Shukla *et al*., 2009).

Insecticidal activity of ethanolic extract of *A. squamosa* was studied on the storage pest, *S. oryzae.* Time required to cause mortality of 50 per cent of test insect (KD_{50}) was recorded to be 23.1 and 11.4 min. at 1 per cent and 5 per cent concentration, respectively, whereas 100 per cent mortality of test insect at the same concentrations was accomplished after 39.6 and 14.5 min. respectively (Kumar *et al.*, 2010).

The leaf extract of *Annona senegalensis* was studied for its insecticidal properties against the seed-beetle, *Caryedon serratus*. The crude methanolic extract of *A. senegalensis* recorded significant mortality of 80.56 per cent both at 0.01 as well as 0.1 g/ml concentration, 72 h after treatment. When different solvent fractions were evaluated at 0.1 g/ ml concentration, methanol fraction recorded significantly higher mortality of 94.4 per cent. However, hexane and acetate fraction recorded 27.78 and 11.11 per cent mortality only, respectively (Sabelle *et al*., 2011).

Nagappan (2012) studied the effect of aqueous as well as ethanolic extract of *Cassia didymobotrya* (*Senna didymobotrya*) against immature stages of *Culex quinquefasciatus*. The ethanolic extract at 150 mg/L concentration recorded 100 per cent mortality of second instar larvae within 24 h, whereas the same mortality was recorded at a higher concentration of 250 mg/L for aqueous extract.

Methanolic extract (1%) of *Annona dioica, A. cacans,* and *A. coriacea* were investigated for their effect on development and reproduction of *Spodoptera frugiperda* which showed significant reduction in per cent viability of larvae as well as pupal biomass, recording 24.00 per cent and 93.4 mg respectively, compared to control (Freitas *et al*., 2014).

An increase in concentration of hexane fraction of *A. calamus* resulted in increased mortality of *Coptotermes curvignathus*, after one day of treatment *i.e.* 5, 10, 15, 20 and 25 per cent concentrations exhibited 32.9, 39.8, 47.7, 54.6, 76.1 and 90.9 per cent mortality, respectively (Adfa *et al.*, 2015).

Bioactivity of ethyl acetate, chloroform-water, chloroform-methanol and methanol fraction of two medicinal plants *Phlomis damascene* and *Ranunculus myosuroides* were evaluated under glass house condition on cucumber seedlings against the cotton whitefly, *Bemisia tabaci*. Among 10 adults released, mean population of 2.66, 2.77, 3.11 and 3.22 were recorded in chloroform-methanol fraction of *P. damascene*, methanol fraction of *R. myosuroides*, chloroform-methanol fraction of *R. myosuroides* and methanol fraction of *P. damascene* respectively, after 72 h (Hammad *et al.*, 2015).

Field efficacy of aqueous extracts of eight plants *viz*., *Citrullus colosynthis*, *Datura innoxia*, *Azadirachta indica*, *Ricinus communis*, *Ferula asafoetida*, *Eucalyptus* sp., *Mimordica charantia* and *Allium sativum* were evaluated against common sucking pests, *Amrasca bigutulla bigutulla*, *Bemisia tabaci* and *Thrips tabaci* in okra. Among the extracts evaluated, neem recorded lowest mean population of jassids, whiteflies and thrips, after seven days of treatment, at 5 per cent concentration (Iqbal *et al*., 2015).

Ethanolic extract of *Acorus calamus* rhizome, when evaluated against *Drosophila melanogaster*, recorded LC50 values of 109.54, 52.51 and 41.11 mg/L, respectively for larva, adult male and female. At highest concentration of 100 mg/L, the male and female recorded significant mortality of 91.11 and 100 per cent, respectively (Kumar *et al*., 2015).

Leaves of *Artemisia herba-alba*, *Eucalyptus camaldulensis* and *Rosmarinus officinalis* were fractionated using petroleum ether, ethanol and distilled water as solvents, against *Myzus persicae*. Etheric extract of *Artemisia herba-alba*, *Eucalyptus camaldulensis* and *Rosmarinus officinalis* had insecticidal action recording 100, 53 and 60 per cent mortality respectively, but the mortality recorded for ethanolic and aqueous extract was not significant (Nia *et al*., 2015).

Longevity as well as fertility of *Euschistus heros* was found to decrease with increase in concentration of seed extract of *Annona mucosa*. The number of eggs laid

per female ranged from 72.70 to 7.10 at 10 to 80 mg $ml^{-1.}$ The seed extract caused 100 per cent mortality of eggs at 40 and 80 mg/ml concentrations (Turchen *et al*., 2016).

Methanolic extracts of *Daphne mucronata*, *Tagetes minuta*, *Calotropis procera*, *Boenninghausenia albiflora*, *Eucalyptus sideroxylon*, *Cinnamomum camphora* and *Isodon rugosus* were evaluated against *Acyrthosiphon pisum*, *Drosophila melanogaster, Tribolium castaneum* and *Spodoptera exigua*. The methanolic extracts (2%) of *Isodon rugosus* and *Daphne mucronata*, which were found to be highly toxic to *Acyrthosiphon pisum* in laboratory assay, were further fractionated on polarity basis using butanol, dichloromethane, hexane and ethyl acetate. The butanol fraction of *I. rugosus* (LC_{50} 18 ppm), ethyl acetate (LC_{50} 68 ppm) as well as dichloromethane fraction (LC_{50} 63 ppm) of *D. mucronata* were found effective among the extracts evaluated (Khan *et al*., 2017).

Effect of *Aegle marmelos* extracts on mortality, oviposition deterrence and inhibition of adult emergence of *Callosobruchus chinensis* was investigated by application of petroleum ether, methanol, ethanol and water extracts at 5 per cent concentration. Petroleum ether extract was more effective than other extracts with 82 per cent mortality, followed by methanol (80%), ethanol (76%) and water extract (74%) after 96 h of treatment. Oviposition deterrence and inhibition of adult emergence was also maximum in petroleum ether extract (Murasing *et al*., 2017).

Five different botanicals, *Azadirachta indica*, *Eucalyptus camaldulensis*, *Nicotiana tabacum*, *Ocimum sanctum* and *Tagetes* sp were screened against dusky cotton bug, *Oxycarenus laetus*. After 72 h of treatment, significantly higher mortality (96%) was recorded by 5 per cent *Nicotiana tabacum*, whereas lowest mortality (52%) was recorded by *Ocimum sanctum* (Saleem *et al.*, 2018).

Bioefficacy of aqueous leaf extract of papaya was investigated against *Aphis* sp. in tomato. Papaya leaf extract at 45 per cent concentration recorded mortality of 55 and 90 percent after 24 and 48 h after treatment respectively. After 48 h, cent per cent mortality of *Aphis* sp. was recorded when treated with 60 per cent extract

(Sunarti, 2019).

Rahayu *et al*. (2020) conducted a study to assess the bioefficacy of ethanolic papaya leaf extracts against standard and field population of German cockroach, *Blattella germanica*. The residue that could cause 90 per cent mortality (lethal residue) ranged from 6.05-8.92 mg/ cm^2 and the time required to reach the same mortality at concentration of 9 mg/ cm^2 ranged from 3.58-5.83 h. Very high level of repellency was recorded (88.99- 94.74%) in all the population by 24 h.

2.1.2. Bioefficacy of botanicals extracts against mite pests

Mites are one of the major and important pests with potential to cause high economic losses. Some important studies pertaining to effect of biorational molecules on mite population are reviewed here.

2.1. 2.1. *Aqueous botanical extracts in mite pest management*

Sarmah *et al.* (2007) investigated the acaricidal activity of the botanicals *Acorus calamus*, *Xanthium strumarium*, *Polygonum hydropiper* and *Clerodendron infortunatum* against tea red spider mite, *Oligonychus coffeae* (Nietner) under laboratory conditions. Strong acaricidal activity was recorded for aqueous extract of *C. infortunatum* with 100 per cent mortality of adult mites at 10 per cent concentration, after 24 h of treatment. It also showed significant mortality of 96 per cent at a lower concentration of 5 per cent. Adult mortality was also observed in *A. calamus* (87.7%), *X. strumarium* (87.2 %), and *P. hydropiper* (77.7%) at 10 per cent concentration, after 24 h of treatment. Egg mortality of 87.09 and 70.62 per cent was recorded by *X. strumarium* and *A. calamus*, respectively at 10 per cent concentration. In field evaluation, extracts of *C. infortunatum* (10%) exhibited 94.5-100 per cent population reduction of *O. coffeae* in seven days of spraying. *Xanthium strumarium* at 10% concentration caused 79.9-90.2 per cent reduction in mite incidence.

Rhizomes of *A. calamus* and leaves of *Vitex negundo* along with neem seed kernel extract (NSKE), pongamia oil, neem oil and two mycopathogens were evaluated for their efficacy against spider mites under polyhouse condition on rose plants. After 10 days of first spraying, neem oil (20 ml/g/l) and *V. negundo* (100 ml/g/l) were found superior to other treatments recording 6.97 and 7.18 mites/ leaflet and similar trend was followed after second and third sprays (Kumar and Nandihalli, 2009).

Neem kernel aqueous extract (NKAE), pongamia kernel aqueous extract (PKAE) and garlic aqueous extract (GAE), each at 5 per cent concentration were evaluated against the red spider mite, *Oligonychus coffeae.* All the botanical extracts recorded significantly higher egg mortality compared to control. Adult mortality reached 100 per cent after 48 h of treatment in GAE and 72 h in PKAE (Roobakkumar *et al.,* 2010).

Roy *et al.* (2011) recorded significant mortality of *Tetranychus neocaledonicus* when treated with 3 per cent aqueous extract of *Eupatorium triplinerve* (61.1%), *Cassia alata* (*Senna alata*) (75%) and *Ocimum tenuiflorum* (82.2%).

Among the different botanicals evaluated against *Phyllocoptruta oleivora*, strongest acaricidal activity was exhibited by aqueous extract of *Jatropha curcas* recording LC_{50} value of 0.80 per cent on par with *Azadirachta indica* (LC_{50} 0.89%), followed by *Mimusops elengi* (LC_{50} 1.06%), *Pometia pinnata* (LC_{50} 1.29%) and *Brucea javanica* (LC_{50} 1.45%). Aqueous extracts of *Barringtonia asiatica* and *Piper* sp. also caused significant mortality (Syahputra and Endarto, 2013).

In a study conducted by Al-Alawi (2014), more than 50 per cent mortality was shown by aqueous extracts of *Phlomis syriaca* (65%), *Achilleae biebersteinii* (64%), *Ruta chalepensis* (53%), *Ballota undulata* (53%), *Astragalus oocephalus* (52%) and *Alkanna strigosa* (52%) against adults of *T. urticae*, two days post treatment. Lethal time estimated for *R. chalepensis* and *Astragalus oocephalus* was found comparable

with acaricide, oomite. In the study, lowest lethal concentration of 8.5 per cent was recorded for *R. chalepensis.*

Radhakrishnan and Prabhakaran (2014) screened ten commonly available weed species for acaricidal activity against *Oligonychus coffeae*. Among the weeds, 5 per cent aqueous extracts of *Allamanda cathartica* and *Conyza bonariensis* showed significantly higher mortality of 100 and 80 per cent, respectively, after 96 h of treatment.

In a laboratory bioassay study, 10 per cent aqueous extract of *Xanthium strumarium* was found to cause highest per cent mortality (89.66%) of *Oligonychus coffeae,* followed by *Swietenia mahagoni* (86.21%), *Polygonum hydropiper* (81.24%), *Datura metel* (69.94%), *Lantana camara* (79.31%) and *Azadirachta indica* (75.86%). The same trend was also observed in field conditions (Al-Mamun *et al.*, 2015).

Ferraz *et al*. (2017) evaluated the efficacy of aqueous extract of juazeiro leaves (*Ziziphus joazeiro*) in the management of the red spider mite, *Tetranychus ludeni,* on cotton. The extract was found effective with high toxicity (LC_{50} 3.54%) and repellency against adult mites of *T. ludeni,* with no phytotoxicity to cotton.

Aqueous extracts of eight plant species were studied for efficacy against adults of *T. urticae* by Hammad *et al.* (2017). Highest mortality was recorded by fruit extract of *Melia azedarach* (1:5), followed by *Melia azedarach* leaf extract (1:5) and *Achillea damascene* (1:5) recording 45.55, 35.55 and 26.66 per cent mortality, respectively.

Premalatha *et al.* (2018) investigated the acaricidal properties of twenty different botanical extracts at ten per cent concentration against *T. urticae.* Among those, highest mortality was observed in *Sesbania grandiflora,* 72 h after of treatment. *Ricinus communis* (85.57%), *Artemisia pallens* (78.90%), *Catharanthus roseus* (77.77%) and *Delonix regia* (76.67%) also exhibited significantly higher mortality. Moderate level of acaricidal property was noticed in botanical extracts of *Achyranthes aspera, Andrographis paniculata, Annona squamosa, Tridax procumbens* and

Allamanda cathartica.

Sathyaseelan *et al.* (2020) compared leaf extracts of some native botanicals with commonly available acaricides for efficacy against *T. utricae* by conducting laboratory bioassay studies. After 48 h of release, maximum mite mortality was recorded by propargite (68.00%) and dicofol (66.00%) followed by *Andrographis* (65.00%), *Vitex* (61.88%) and pongamia (60.00 %). The botanical extracts evaluated were found comparable with the acaricides.

2.1.2.2. *Solvent extracts of botanicals in mite pest management*

The ethanol extracts of *Artemisia leucodes* and *Eruca sativa* showed combined values of repellency and adulticidal effect against *T. cinnabarinus* of 82-90 per cent and 83 per cent respectively at 5000 µg/ml concentration. However, extract of *Sinapis alba* and *Artemesia herba alba* presented only moderate acaricidal activity (Azaizeh *et al*., 2007).

Methanolic extracts of *Eupatorium triplinerve*, *Ocimum tenuiflorum* and *Cassia alata* at 3 per cent resulted in 74.4, 93.3 and 97 per cent mortality of adult *T. neocaledonicus*, respectively (Roy *et al*., 2011).

The ethanolic extracts of whole plant parts of *Ambrosia maritimal*, leaves of *Duranta plumeria* and seeds of *Cuminum cyminum* were evaluated for acaricidal properties against eggs and adults of *Oligonychus afrasiaticus* at different concentrations. Among the extracts, *A. maritimal* recorded significant mortality of adult mites recording 93.33 per cent at 1×10^5 ppm concentration, after one day of treatment. *Ambrosia maritima*, *D. plumeria* and *C. cyminum* recorded 87.33, 70.67 and 30.67 per cent mortality of eggs, respectively. Also, *A. maritima* recorded the least LC_{50} value of 47.16 ppm and highest toxicity index of 100% (Fetoh and Al-Shammery, 2011).

Ethanolic extracts of *Allium sativum, Rhododendron luteum, Helichrysum arenarium, Veratrum album* and *Tanacetum parthenium* were evaluated for efficacy against *T. urticae.* The ethanol extract of *T. parthenium* at 12 per cent concentration recorded the highest adult mortality (88%) as well as larval mortality (82%) when compared with the rest of the botanicals (Erdogan *et al*., 2012).

Methanolic leaf extracts of *Anisosciadium orientale, Scaligeria meifolia, Trigonella elliptica* and *Dodonaea viscosa* were evaluated for ovicidal action against *T. urticae,* however none of the extracts caused more than 50 per cent mortality (Ghaderi *et al.,* 2013).

Syahputra and Endarto (2013) studied the acaricidal properties of some ethanolic botanical extracts against citrus rust mite, *Phyllocoptruta oleivora* and citrus red mite, *Panonychus citri*. Ethanolic seed extract of *Mimusops elengi, Pometia pinnata, Azadirachta indica, Barringtonia asiatica* and fruit extract of *Brucea javanica* showed 100 per cent mortality of adult *P. oleivora.* Cent per cent mortality was also exhibited by seed extract of *Mimusops elengi* against *Pa. citri*. Ethanolic seed extract of *Jatropha curcas* (96.4%), seed extract of *Piper sp*. (95.5%), fruit peel extract of *Pseuderanthemum graciliflorum* (93.1%), leaf extract of *A. indica* (92.9%), leaf extract of *P. graciliflorum* (91.7%) also showed significant mortality against *P. oleivora.*

Acetone extract of *Azadirachta indica, Vitex negundo, Clerodendrum infortunatum, Butea monosperma* and *Pongamia pinnata* each at 2.5 per cent concentration, along with bio-pesticide I (combination of tobacco leaves, garlic extract, *Acorus calamus*, red chilli, neem extract and neem oil) and bio-pesticide II (combination of pongamia extract and neem oil) each at 3 per cent concentration, were evaluated for acaricidal activity against *Tetranychus ludeni*. The combination of pongamia extract and neem oil recorded highest mortality of 78.89 per cent, while pongamia extract alone recorded 50.00 per cent mortality only. All the extracts evaluated showed 100 per cent mortality by fourth day of treatment. Among the extracts, *P. pinnata* showed highest repellency (87.50%), followed by *C. infortunatum*

and *V. negundo* (Gupta and Mondal, 2015).

Prasad and Gupta (2016) evaluated bio effectiveness of *V. negundo, Clerodendrum viscosum* and *Polyalthia longifolia*, at 2.5 and 5 per cent concentrations against *Brevipalpus essigi*. *Clerodendron viscosum* (5%) recorded highest mean mortality of 88.66 per cent after 96 h.

Salma *et al.* (2017) evaluated mortality and repellency of methanolic extracts of *A. indica, V. negundo, C. infortunatum, B. monosperma* and *P. pinnata* against *T. ludeni,* infesting *Rauwolfia serpentina* at 2.5 per cent concentration. Among the extracts, *V. negundo* recorded highest mortality of 95.00 per cent, 24 h after treatment. After four days all other treatments recorded mortality of 70.00 per cent and above. Highest significant repellency 80 per cent was recorded in *V. negundo*.

Nandini and Srinivasa (2018) conducted a laboratory study to evaluate the ovicidal deterrence, repellency and mortality caused by leaf extracts of *Vitex* spp. on okra red spider mite, *Tetranychus macfarlanei*. Methanol extract of *Vitex altissima* and *Vitex negundo* showed reduced mean number of eggs laid per mite (3 days period). Among different organic solvents evaluated, methanol extract was found effective, with maximum repellency observed in *Vitex trifolia*. Methanol extract of *V. peduncularis* showed maximum mortality, recording 79 per cent.

Numa *et al.* (2018) examined ethanolic extracts of certain native Colombian plants for their acaricidal effects against *T. urticae* at 0.06 per cent concentration. Maximum adult mortality of 83.33 per cent was observed in *Copaifera officinalis,* after 96 h of treatment. Extracts of *Bowdichia virgilioides, C.officinalis* and *Anadenanthera peregrina* recorded mortality of 60 per cent and above. Fecundity of the mites was adversely affected by ethanolic extracts of *Cnidoscolus aconitifolius, A. peregrine* and *C. officinalis.*

Ethanolic leaf extract of *Tagetes patula* was screened against egg, larva and adult of *T. urticae.* Four days after treatment, leaf extract at 8 per cent concentration

recorded only 4.17 per cent hatchability when compared with control (69.18%). Larval mortality of 100 per cent was observed after 6 days of treatment. After 24 h of treatment, 5 per cent extract recorded highest mean mortality of adult mites (88.9%) and LC_{50} was found to be 0.99 per cent. Sublethal concentration (0.5%) of ethanolic extract of *Tagetes patula* was found to be an effective repellent against *T. urticae* (Ismail *et al.,* 2019).

Among the six plant extracts evaluated *viz*., *Arachis hypogaea, Perilla frutescens, Lilium brownii* var. *viridulum, Nelumbo nucifera, Phragmites australis* and *Platycladus orientalis,* the stem and leaf extract of *Arachis hypogaea* recorded the highest kill (99.61%) against *Tetranychus cinnabarinus*, at 15,000 mg/L concentration. Even the lower concentrations, *i.e*. 5,000 and 6,000 mg/L were found promising during the laboratory bioassay (Liu *et al.,* 2019).

2.1.2.3. Bioefficacy of solvent fractions of botanical extracts against mite pests

Efficacy of different fractions of green marine algae (*Codium* sp.) *viz.,* petroleum ether, diethyl ether, chloroform, acetone and ethyl alcohol were evaluated against egg as well as adult of *T. urticae*. Among the fractions, petroleum ether fraction (4g/ml) recorded significantly higher mortality of adult mite (82.50%). But even at higher concentrations, none of the green marine algal fractions showed more than 50 per cent mortality of eggs (Amer *et al.*, 1991).

El-Khayat *et* al. (2014) investigated the toxicity and repellency of *Aloe vera* fractions (hexane, acetone, ethanol and water) against adult females of *Tetranychus urticae*. Acetone fraction recorded least LC_{50} value of 581.92 ppm and highest toxicity index of 100 per cent. Acetone fraction was also found to reduce the longevity as well as fecundity of the mite, with deterrent index of 26.12 per cent. However, significant repellency was recorded by hexane fraction compared to other fractions.

Dichloromethane and hexane fraction of dry seed extracts of *Leucaena glauca* at 9 per cent concentration was found effective against *T. urticae* causing mortality of 92.9 per cent and 100 per cent respectively after 24 h of treatment. Even at lower concentrations (10000 ppm), dichloromethane and hexane fractions showed significant mortality of 98 and 80 per cent respectively. Reduction in number of eggs laid per female at 10000 ppm was noted to be 100 per cent (Auamcharoen and Chandrapatya, 2015).

Fernandes *et al.* (2017) conducted a study to evaluate the bioefficacy of methanol and hexane extracts of *Annona vepretorum* leaves against *T. urticae*. The toxicity of methanol extract was evident, with higher mortality in topical and residual application, with LC_{50} value of 10.96 (mg L^{-1}) compared to the residual application alone, with LC_{50} value of 22.07 (mg L^{-1}). Within 24 h of treatment, mortality of adult mites recorded 93 per cent in topical and residual application whereas 65 per cent in residual application alone.

Among different solvents evaluated, petroleum ether partition of ethanolic extract of *Arachis hypogaea* was found effective against *T. cinnabarinus* in bioassay guided fractionation. On further fractionation, ninth fraction of petroleum ether partition showed highest activity. The active compound was isolated by column chromatography and identified as palmitic acid through mass spectroscopy and nuclear magnetic resonance analyses (Liu *et al.*, 2019).

2.2. SAFETY OF BOTANICAL EXTRACTS TO NATURAL ENEMIES OF INSECT AND MITE PESTS

Aqueous leaf extracts of *Clerodendron inermae* and *Vitex negundo* were evaluated at 5 per cent concentration, along with neem seed kernel extract (5%), neem gold (0.15 EC), some acaricides and bioagents against natural enemies of yellow mite, *Polyphagotarsonemus latus* namely, *Coccinella septempunctata* and *Amblyseius* sp. *Vitex negundo* and *C. inermae* resulted in population reduction from 0.28 to 0.20 and 0.30 to 0.25 mites per leaf, respectively, seven days after treatment application.

However, none of the botanical extracts caused reduction in population of *C. septempunctata* (Smitha and Giraddi, 2006).

The plant extracts of *Xanthium strumarium, Acorus calamus* and *Polygonum hydropiper* at 10 per cent concentration resulted in significant reduction in the feeding potential of the predatory coccinellid, *Stethorus gilvifrons* after 24 h of treatment. However, the plant extracts did not cause any mortality of *S. gilvifrons* until 14 days (Sarmah *et al*, 2007).

A combination of methanolic extracts of *Piper retrofractum* and *Annona squamosa* (RS) as well as *Aglaia odorata* and *A. squamosa* (OS) were evaluated against the hymenopteran parasitoids, *Diadegma semiclausum* and *Eriborus argentiopilosus*. The botanical pesticide mixture, RS (0.1 %) did not affect the level of parasitization by any of the parasitoids after 10 weeks of treatment, but the botanical pesticide mixture, OS (0.1 %) reduced parasitization of *E. argentiopilosus* (Dadang *et al.*,2009).

Compatibility of different solvent extracts of *Syndrella nodiflora*, *Premna tomentosa*, *Vitex negundo*, *Ipomea carnea*, *Pteridium aquilinum* and *Annona squamosa* were studied on the growth of entomopathogenic fungus, *Beauveria bassiana* following poisoned food technique. Maximum inhibition of growth was observed in *S. nodiflora* benzene extract recording 63.8 per cent. Significant inhibition of 57.1 per cent was also observed in water extract of *A. squamosa* (Sahayaraj *et al.*, 2011).

Sandrine *et al*. (2013) studied the antifungal action of ethanolic extract of *Ageratum conyzoides, Callistemon citrinus*, *Cymbopogon citratus* and *Ocimum gratissimum* against pathogenic fungus, *Acremonium apii* and *Colletotrichum dematium*. Highest significant inhibition against the fungus, *A. apii* and *C. dematium* was exhibited by 10,000 ppm extract of *C. citrinus*, recording 77.68 and 97.16 per cent, respectively.

The active aqueous extracts of *Jatropha curcas, Azadirachta indica, Mimusops elengi*, *Pometia pinnata* and *Brucea javanica* were tested against the mite predator, *Harmonia axyridis*. None of the extracts at 5 per cent concentration caused mortality of the predator (Syahputra and Endarto, 2013).

Safety of aqueous botanical extracts of *Polygonum hydropiper*, *Xanthium strumarium*, *Datura metel*, *Lantana camara*, *Swietenia mahagoni* and *Azadirachta indica* was evaluated against two important predators of *Oligonychus coffeae at* 2.5, 5.0 and 10.0 per cent concentrations. Even at higher concentration, none of extracts caused mortality of the adult predators, *Stethorus gilvifrons* and *Oxyopes* sp. (Al-Mamun *et al.*, 2015).

Dutta *et al*. (2016) conducted a study to investigate the effect of buprofezin, diafenthiuron, lufenuron, indoxacarb and azadirachtin against the mustard aphid, *Lipaphis erysimi*, coccinellid predators and foraging honeybees. Seven days after treatment, azadirachtin showed least per cent reduction of coccinellid predator recording 29.38 per cent, whereas the insecticides indoxacarb, lufenuron, buprofezin and diafenthiuron recorded 63.69, 55.42, 52.63 and 52.24 per cent reduction, respectively.

Fresh infusions of oregano, laurel and rosemary leaf essential oil were evaluated against the fungus, *Acremonium* sp. The oregano leaf essential oil recorded 100 per cent growth inhibition of the fungus at 20 per cent concentration, whereas essential oil from rosemary and laurel leaves recorded inhibition of 63 and 44 per cent, respectively (Racowski *et al*., 2016).

The toxicity of ethanolic extracts of *Salvia officinalis* (sage) and *Rosmarinus officinalis* (rosemary) were evaluated against two important predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis*. After 72 h, the highest concentration (12 ml/L) evaluated showed 33.3 and 62.5 per cent mortality of *N. longispinosus* when treated with *S. officinalis* and *R. officinalis* extracts, respectively. However, in the case of *P. persimilis*, significantly higher mortality of 33.3 and 38.4

per cent was shown by the extracts of *S. officinalis* and *R. officinalis,* respectively at the same concentration. The extracts were also evaluated against the egg stage of the predatory mites. Treatment *R. officinalis* at 12 ml/L caused significantly higher egg mortality of 51.1 per cent against *N. californicus*, whereas, *S. officinalis* recorded mortality of 16.6 per cent only. Both the extracts were found relatively safer to the eggs of *P. persimilis,* each recording mortality of 18.6 per cent (Salman *et al.,* 2018).

Materials and methods

3. MATERIALS AND METHODS

The present study entitled "Bioefficacy of botanicals against the spider mite, *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)" was conducted in the Acarology laboratory, Department of Agricultural Entomology, College of Horticulture, KAU, Vellanikkara during 2018-2020. The objectives of the study were to evaluate the efficacy of botanical extracts against *Tetranychus truncatus*, to identify bioactive solvent fractions of promising plant extracts against *T. truncatus* and to test the safety of bioactive fractions to the predatory mite, *Neoseiulus longispinosus* and the acaropathogen, *Acremonium zeylanicum*. The materials used and methods employed for conducting various experiments based on the objectives set forth in the study are presented herewith.

3.1 BIOEFFICACY OF BOTANICALS AGAINST *TETRANYCHUS TRUNCATUS*

Ten plants *viz*., *Acorus calamus L.*, *Bacopa monnieri* (L.) Pennell, *Quassia indica* Gaern, *Eucalyptus* sp., *Lantana camara* L., *Aegle marmelos* (L.) Correa, *Annona squamosa L.*, *Vitex negundo* L., *Carica papaya* L. and *Ocimum sanctum* L. were selected for evaluating their efficacy against *Tetranychus truncatus* Ehara.

3.1.1. Collection and preservation of botanicals

The plants *Quassia indica*, *Eucalyptus* sp., *Lantana camara*, *Aegle marmelos* and *Carica papaya* were collected fresh from their natural habitats from different localities of Kerala during the study. Rest of the botanicals was procured from ayurvedic herbal store located in Thrissur market. The details of the locality of collection of the botanicals as well as plant parts used for the study are furnished in Table 1. The botanicals were shade dried in the insectary building of the Department of Agricultural Entomology (Plate 1-4.) and finely powdered using an electric mixer. Each plant material was sieved and stored separately in air tight containers (Plate 5) at 4 º C in a refrigerator until further used.

Table 1. **Botanicals screened for acaricidal activity against *Tetranychus truncatus***

Sl. No	Scientific Name	Common/ vernacular name	Family	Plant part used	Location of collection
1.	*Acorus calamus*[*]	Sweet flag	Acoraceae	Rhizome	$10^0$31'16.918 "N, $76^0$13'6.14"E Thrissur
2.	*Bacopa monnieri*[*]	Water hyssop	Plantaginaceae	Whole plant	$10^0$31'16.918"N, $76^0$13'6.147"E Thrissur
3.	*Quassia indica*	Niepa bark tree	Simaroubaceae	Leaves	$10^0$32'50.886"N, $76^0$16'56.147"E Thrissur
4.	*Eucalyptus* spp	Eucalyptus	Myrtaceae	Leaves	$11^0$48'32.969"N, $76^0$59'51.838"E Wayanad
5.	*Lantana camera*	Wild sage	Verbenaceae	Whole plant	$11^0$48'58.873"N, $75^0$59'41.137"E Wayanad
6.	*Aegle marmelos*	Bael	Rutaceae	Leaves	$10^0$33'20.456"N, $76^0$17'6.224"E Thrissur
7.	*Annona squamosa*[*]	Custard apple	Annonaceae	Seeds	$10^0$31'16.918"N, $76^0$13'6.147"E Thrissur
8.	*Vitex negundo*[*]	Chaste tree	Lamiaceae	Leaves	$10^0$31'16.918"N, $76^0$13'6.147"E Thrissur
9.	*Carica papaya*	Papaya	Caricaceae	Leaves	$11^0$48'18.969"N, $76^0$1'3.61"E Wayanad
10.	*Ocimum sanctum*[*]	Holy basil	Lamiaceae	Leaves	$10^0$31'16.918"N, $76^0$13'6.147"E Thrissur

* Indicate botanicals collected from market


Plate 1a. ***Lantana camara***


Plate 1b. ***Aegle marmelos***


Plate 1c. ***Eucalyptus*** **sp.**

Plate 1. Fresh and dry plant parts 1a. ***Lantana camara*** **1b.** ***Aegle marmalos*** **1c.** ***Eucalyptus*** **sp.**

Plate 2a. *Vitex negundo*

Plate 2b. *Ocimum sanctum*

Plate 2c. *Quassia indica*

Plate 2. Fresh and dry plant parts 2a. *Vitex negundo* 2b. *Ocimum sanctum* 2c. *Quassia indica*

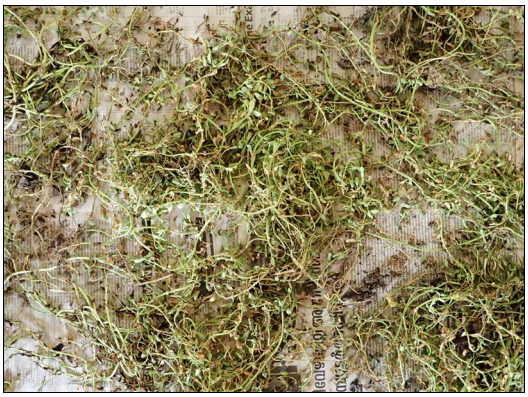

Plate 3a. ***Bacopa monnieri***

Plate 3b. ***Carica papaya***

Plate 3c. ***Annona squamosa*** **Plate 4.** ***Acorus calamus***

Plate 3. Fresh and dry plant parts 3a. ***Bacopa monnieri*** **3b.** ***Carica papaya*** **3c.** ***Annona squamosa***

Plate 4. Dried ***Acorus calamus*** **rhizome**

Plate 5a. Pulverizing botanicals

Plate 5b. Seiving

Plate 5c. Storage

Plate 5. Preparation and storage of botanicals

3.1.2. Mass culture of *Tetranychus truncatus*

Gravid females collected from the nucleus culture of *T. truncatus* maintained in the Acarology laboratory of AINPAA (All India Network Project on Agricultural Acarology) were used to initiate the mass culture. The females were released onto mulberry leaves, placed upside down on plastic trays ($40\times28cm^2$) lined with moistened sponge (Plate 6). The leaves were replaced at an interval of three to four days, by positioning the old leaf harboring mites over a fresh leaf, so that the mites got transferred to new leaf naturally.

3.1.3. Laboratory bioassay of botanical extracts against *Tetranychus truncatus*

Acaricidal activity of crude extracts **(**aqueous and methanol) of the selected botanicals were evaluated separately on adults and eggs of *T. truncatus* at different concentrations.

3.1.3.1. Bioassay of aqueous extract of botanicals against Tetranychus truncatus

Aqueous extracts of different botanicals were evaluated for efficacy against *T. truncatus* at three different concentrations (5, 7.5 and 10 %).

3.1.3.1.1. Preparation of aqueous botanical extract

Aqueous extracts of the botanicals were prepared at three different concentrations (5, 7.5 and 10 %) by weighing 5, 7.5 and 10g of the powdered plant material respectively, and soaking separately in 100 ml of distilled water in 250ml conical flasks. The mixture was shaken for 8 hours continuously in rotary shaker at 200 rpm and filtered using muslin cloth after 24 h (Plate 7).

3.1.3.1.2. Ovicidal effect of aqueous extract of botanicals

The effect of aqueous extract (5, 7.5 and 10%) of the ten different botanicals was evaluated against the eggs of *T. truncatus* by following topical application method (Yadav, 2018). The experiment was laid out in Completely Randomised

Design (Plate 8).

Mulberry leaf bits (5×5 cm^2) were placed in Petri plates (200×30mm) lined with moistened cotton. Ten gravid females were released per leaf bit and allowed to lay eggs. The gravid females were removed after 24 h in order to obtain one day old eggs. Up to twenty five eggs laid per leaf bit were counted under a stereomicroscope and excess eggs were removed carefully by pricking with a needle. Leaf bits containing *T. truncatus* eggs were sprayed with appropriate treatments using a hand atomizer (2ml/bit) (Plate 9). Three replications were maintained for each concentration of the extracts. Eggs on mulberry leaves sprayed with water alone served as untreated control. The hatchability of eggs after 24, 48, 72, 96 and 120 h of spraying was recorded and per cent mortality of egg was calculated.

3.1.3.1.3. Adulticidal effect of aqueous extract of botanicals

The effect of aqueous (5, 7.5 and 10%) extracts of different botanical extracts against the gravid females of *T. truncatus* was evaluated by leaf dip method (Yadav, 2018).

Mulberry leaf bits (7×7 cm^2) were dipped in beaker (100 ml) containing botanical extract for 60 sec (Plate 10). The leaf bits were subjected to air drying for 20 minutes. Twenty five gravid females were released to each leaf bit after placing it in a Petri plate lined with moistened cotton. Each leaf bit was padded with water soaked cotton along the margins to restrict the mites from leaving the treated leaf bit. The experiment was laid out in Completely Randomised Design and three replications were maintained per treatment. Mortality of mites was recorded 24, 48, 72, 96 and 120 h after treatment and per cent mortality was calculated.

3.1.3.2. *Bioassay of methanol extract of botanicals against Tetranychus truncatus*

Methanol extracts of different botanicals were evaluated for efficacy against *T. truncatus* at two different concentrations (1 and 2%).

Plate 6a. Mass culture of *T. truncatus*

Plate 6b. Adults of *T. truncatus*

Plate 6c. Eggs and nymphs of *T. truncatus*

Plate 6. Mass culture of *Tetranychus truncatus* on mulberry leaves

Plate 7a. Shaking in rotary shaker **Plate 7b. Filtration**

Plate 7. Preparation of aqueous extracts

Plate 8. Experimental setup for ovicidal bioassay of aqueous extracts

3.1.3.2.1. *Preparation of methanol extract*

Direct methanol extract was prepared by weighing 100g of powdered plant material, taken in 500ml conical flask and 300ml of methanol was added (3X), followed by shaking for 48 h. The plant suspension was filtered using a filter paper (sonar sheet) and the solvent was evaporated under vacuum using rotary evaporator at 40º C (Plate 11). The resulting residue was dried, and 1 and 2 per cent of methanol extracts were prepared, respectively by dissolving 1g and 2g of the residue separately in 100ml of distilled water.

3.1.3.2.2. Ovicidal effect of methanol extract of botanicals

The effect of methanol extracts (1 and 2 %) of the 10 different botanicals were evaluated against the eggs of *T. truncatus* by following topical application method (Yadav, 2018) as described in 3.1.3.1.2. The experiment was laid out in Completely Randomised Design with 21 treatments and three replications (Plate 12). The hatchability of eggs after 24, 48, 72, 96 and 120 h of spraying was recorded and per cent mortality of egg was calculated.

3.1.3.2.3. *Adulticidal effect of methanol extract of botanicals*

The effect of methanol extracts (1 and 2%) of different botanicals against the gravid females of *T. truncatus* was evaluated by leaf dip method (Yadav, 2018) as described in 3.1.3.1.3. The experiment was laid out in Completely Randomised Design. Three replications were maintained per treatment. Mortality of mites was recorded 24, 48, 72, 96 and 120 h after treatment and per cent mortality was calculated.

3.1.4. *Data analysis*

The data on per cent mortality of eggs and adults treated with aqueous as well as methanol extracts were subjected to analysis of variance using the software, Web Agri Stat Package 2.0 (WASP 2.0), developed by Central Coastal Agricultural Research Institute - Goa, India.

3.2. IDENTIFICATION OF BIOACTIVE SOLVENT FRACTION OF PROMISING PLANT EXTRACTS

One of the promising botanicals, *Acorus calamus*, found effective against the gravid females of *T. truncatus* based on the laboratory bioassay studies, was further subjected to bioassay guided fractionation, to identify the bioactive solvent fraction in it.

3.2.1. Activity guided solvent fractionation by liquid-liquid partitioning

The powdered rhizome of *Acorus calamus* was further fractionated sequentially based on the polarity of solvents into three fractions (Plate 13) using hexane (non polar), chloroform (medium polar) and methane (highly polar) as solvents**.** The efficacy of different solvent fractions against *T. truncatus* was evaluated in the laboratory**.**

3.2.1.1. *Solvent fractionation by liquid-liquid partitioning*

Pulverized plant material was weighed and extracted using hexane (3X) by placing it in a rotary shaker for 48 h. The solution was then filtered and the filtrate concentrated by a rotary evaporator at 40^0C to obtain the hexane extract. The residue obtained during filtration was re-extracted sequentially using chloroform followed by methane, using the same procedure as hexane extraction, to obtain chloroform extract and methanol extract, respectively (Auamcharoen and Chandrapatya, 2015).

3.2.1.2. *Bioactivity of solvent fractions*

Efficacy of hexane, chloroform and methanol fractions of *A. calamus* each at three different concentrations (0.3, 0.5 and 0.7%) obtained in 3.2.1.1 were evaluated separately on eggs and adults of *T. truncatus*. The experiment was laid out in Completely Randomised Design with three replications for each treatment.

Plate 9. Topical application for ovicidal bioassay

Plate 10. Leaf dip method for adulticidal bioassay

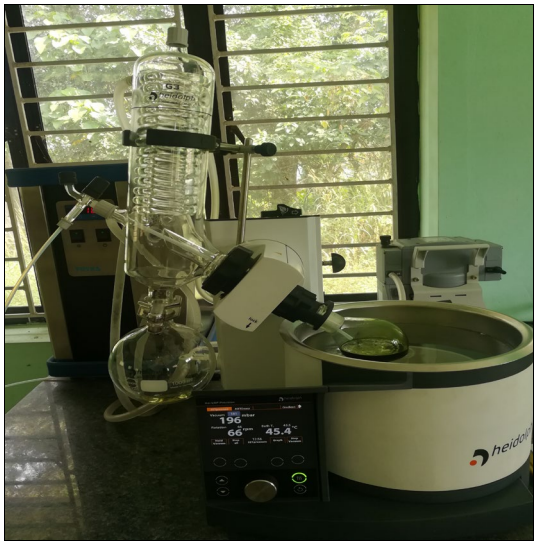

Plate 11. Solvent evaporation using rotary evaporator

Plate 12. Experimental set up for ovicidal bioassay of methanol extracts

3.2.1.2.1. *Ovicidal bioassay*

Ovicidal effect of different fractions of *A. calamus*, was evaluated against the eggs of *T. truncatus* by following topical application method as described in 3.1.3.2. The hatchability of eggs after 24, 48, 72, 96 and 120 h of treatment was recorded and per cent mortality of egg was calculated (Plate 14).

3.2.1.2.2. *Adulticidal bioassay*

Adulticidal effect of different fractions of *A. calamus*, was evaluated against *T. truncatus* by following leaf dip method as described in 3.1.3.3. Mortality of mites was recorded 24, 48, 72, 96 and 120 h after treatment and per cent mortality was calculated.

3.2.1.3. *Data analysis*

The data on per cent mortality of eggs and adults of *T. truncatus* was subjected to analysis of variance using the software, Web Agri Stat Package 2.0 (WASP 2.0), developed by Central Coastal Agricultural Research Institute - Goa, India.

3.2.2. Evaluation of botanical extracts against *Tetranychus truncatus* in polyhouse

A **f**ield experiment was conducted to evaluate the efficacy of the sequential methanol fraction of *A. calamus* against *T. truncatus* on cucumber in the polyhouse of AINPAA, Department of Agricultural Entomology, during May- June, 2020. Two concentrations of sequential methanol fraction of *A. calamus* (0.3 and 0.5%), one promising aqueous extract identified in the laboratory bioassay studies viz., *Annona squamosa* (7.5%), neem oil emulsion (2%), horticultural mineral oil (2.5%) and an acaricide, spiromesifen 240SC (100g a.i/ha) were evaluated in the study. Water spray was considered as control treatment. The experiment was laid out in Completely Randomized Design with seven treatments, each replicated thrice (Plate 15).

The cucumber variety - KPCH 1 was used for the study. The seeds were collected from the Department of Vegetable Science, College of Horticulture and sown in pro trays. Plants were transplanted at two leaf stage (one week after sowing) with a spacing of 60 and 30 cm between rows and plants, respectively. Mites were released on three leaves of 25 days old cucumber plants at the rate of 25 active mites per leaf by stapling mite infested mulberry leaf bit of size $3cm^2$ each on the top, middle as well as bottom leaf of cucumber plant (Plate 16). Treatments were imposed 15 days after the release of mites using a hand sprayer (Plate 17).

For recording the mite population in treatments, five plants were selected randomly from each treatment replication. Number of mites (both egg and active stages) was recorded from three windows of $1cm^2$ area, each from three leaves per plant representing the top, middle and bottom canopy of the plant (Plate 18). Pre-treatment count one day prior to treatment and post treatment count of mite at 1, 3, 7, 10 and 14 days after treatment application were recorded using a hand lens.

3.2.2.1. *Data analysis*

Data on mean number of mites per cm^2 leaf area before and after treatments were subjected to analysis of variance using the software, Web Agri Stat Package 2.0 (WASP 2.0), developed by Central Coastal Agricultural Research Institute - Goa, India.

3.3. EVALUATION OF SAFETY OF BOTANICAL EXTRACT AGAINST THE PREDATORY MITE, *NEOSEIULUS LONGISPINOSUS*

The effect of the most promising botanical fraction, identified from studies on bioassay guided fractionation was evaluated on egg and adults of the predatory mite, *Neoseiulus longispinosus* along with neem oil and horticultural mineral oil.

Plate 13a. Hexane fraction

Plate 13b. Chloroform fraction

Plate 13c. Methanol fraction

Plate 13. Solvent fractions of *Acorus calamus*

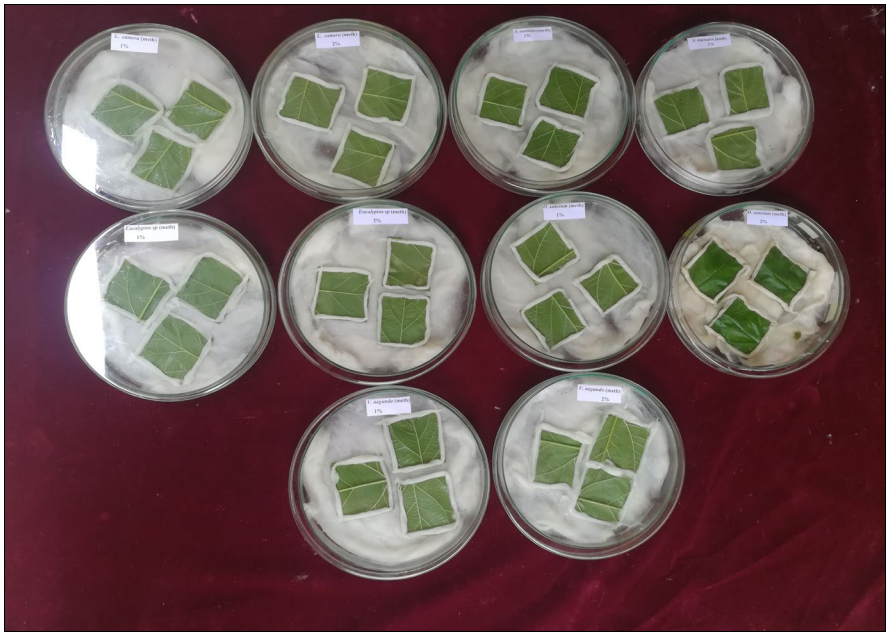

Plate 14. Experimental set up for ovicidal bioassay of solvent fractions of *Acorus calamus*

Plate 15. Experiment in polyhouse

Plate 16. Release of mites on cucumber plant

Plate 17. Spraying of botanical extracts

Plate 18. Recording mite population using window count

3.3.1. Maintaining laboratory culture of *Neoseiulus longispinosus*

Predatory mite culture was maintained in the Acarology laboratory of AINPAA, on the prey mite, *T. truncatus*. For this, gravid females of *T. truncatus* were released on mulberry leaves placed on moistened synthetic absorbent sponge in plastic trays. Four days after the release, when the prey population had established, six gravid females of *N. longispinosus* were released onto prey culture. As the prey population decreased, to replenish the prey, leaf bits containing prey mites were placed on the mulberry leaves regularly. Old leaves were replaced by placing them over new leaves on the sponge so that both the prey the predatory mite got transferred to the new leaf naturally.

3.3.2. *Laboratory bioassay*

The sequential methanol fraction of *A. calamus* was evaluated for its safety to the egg and adult of the predatory mite *N. longispinosus*, separately, at three different concentrations. The experiment was laid out in Completely Randomized Design with six treatments and three replications. The treatments included three different concentrations of sequential methanol fraction of *A. calamus* (0.3, 0.5 and 0.7 %), neem oil emulsion (2%) horticultural mineral oil (2.5%) and an untreated control.

3.3.2.1 *Ovicidal bioassay*

Ovicidal effect of methanol fraction of *A. calamus*, neem oil emulsion and horticultural mineral oil were evaluated against the eggs of *T. truncatus* by following topical application method.

Five gravid females of the predatory mite, *N. longispinosus* were released onto mulberry leaves with prey mite, *T. truncatus*. Leaves were kept in Petri plates lined with moistened cotton and a thin layer of wet cotton was provided around the leaf margin to prevent escape of mites. After 24h of treatment, all predatory mites were removed and only six eggs of predatory mites were retained in each leaf. Three

concentrations of sequential methanol fraction of *A. calamus*, neem oil emulsion and horticultural mineral oil evaluated were sprayed on the eggs of *Neoseiulus longispinosus* at specified concentrations. Observations on hatchability of eggs were made at 24, 48 and 72 h after treatment and per cent mortality was calculated.

3.3.2.2 *Adulticidal bioassay*

The effect of treatments on the adults of *N. longispinosus* was studied following leaf dip bioassay method. Six adults of *N. longispinosus* were released onto the treated leaves kept in Petri plates lined with moistened cotton and a thin layer of wet cotton was provided around the leaf margin to prevent escape of mites. Adequate prey mites were provided regularly as food for the predatory mite. Mortality of the adult *N. longispinosus* at 24, 48 and 72 h after application of treatment was recorded and per cent mortality was calculated.

3.3.3. *Data analysis*

The data on per cent mortality of mites was subjected to analysis of variance using the software Web Agri Stat Package 2.0 (WASP 2.0), developed by Central Coastal Agricultural Research Institute - Goa, India.

3.4. EVALUATION OF SAFETY OF BOTANICAL EXTRACT TO THE ACAROPATHOGEN, *ACREMONIUM ZEYLANICUM*

The effect of the most promising sequential methanol fraction of *A. calamus* (0.3, 0.5 and 0.7 %), was evaluated along with neem oil (2%) and horticultural mineral oil (2.5 %) on growth of the acaropathogen, *A. zeylanicum* following poison food technique. The experiment was laid out in Completely Randomized Design with six treatments and three replications.

3.4.1 Maintenance of *Acremonium zeylanicum* culture

Nucleus culture of *A. zeylanicum* maintained in the Acarology laboratory, AINPAA was used in the study. *Acremonium zeylanicum* was cultured in Sabouraud Dextrose Agar with the addition of 2 per cent Yeast extract (SDAY) in slants and on Sabouraud Dextrose in conical flasks (SDY).

Thick fungal mat of *A. zeylanicum* was ground using a sterilized mixer, with Tween 80 (0.05%) added and vortexed. The suspension was then filtered through a double layered muslin cloth and sprayed on *T. truncatus* culture maintained on mulberry leaves. The infected mites were transferred to humid chamber and fungus was re-isolated from the moribund mites. This procedure helped to maintain the virulence of the acaropathogen.

Both the media and broth was autoclaved at 121º C temperature and 15 PSI pressure for 20 minutes. The media was poured into Petri plates @ 20 ml per plate, followed by inoculation of fungus under aseptic condition and incubated at room temperature. Pure culture was maintained by frequent sub culturing into slants and Petri plates.

3.4.1 Sensitivity of *Acremonium zeylanicum* to botanical fraction

The sequential methanol fraction of *A. calamus* (0.3 and 0.5 %), neem oil (2%) and HMO (2.5%) were evaluated in the laboratory for their inhibitory effect on *Acremonium zeylanicum* following poisoned food technique (Mohana and Raveesha, 2007). The treatments at specified concentrations were mixed separately with 100ml SDAY media and plated in aseptic condition. Prior to mixing, neem oil was disinfected under UV light and for proper mixing in media, Triton X_{100} (non-ionic detergent) @ 0.2 per cent was added. Circular mycelial disc of diameter 5mm from *A. zeylanicum* fresh culture maintained in the Acarology laboratory, was cut using a sterile cork borer and placed in the centre of the poisoned media in Petri plate. The experiment was laid out Completely Randomised Block Design with three

replications. Media which was not poisoned served as control treatment. Petri plates were sealed using parafilim and incubated at room temperature. Observation on growth of *A. zeylanicum* was taken until the control plate showed full growth (90mm). The per cent inhibition on growth of pathogen was calculated using the formula given by Vincent (1947).

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

where,

C = Diameter of fungal growth in control

T = Diameter of fungal growth in treatment

Results

4. RESULTS

Results of the study on bioefficacy of botanicals against the red spider mite, *Tetranychus truncatus*, Ehara (Prostigmata: Tetranychidae), based on the experiments conducted in the laboratory and polyhouse of All India Network Project on Agricultural Acarology, Department of Agricultural Entomology are presented in this chapter.

4.1. LABORATORY BIOASSAY OF BOTANICAL EXTRACTS AGAINST *TETRANYCHUS TRUNCATUS*

Aqueous as well as methanol extracts of ten botanicals viz., *Acorus calamus, Bacopa monnieri, Quassia indica, Eucalyptus* sp., *Lantana camara, Aegle marmelos, Annona squamosa, Vitex negundo, Carica papaya* and *Ocimum sanctum* were evaluated at different concentrations for acaricidal properties against egg and adult of *T. truncatus* under laboratory condition.

4.1.1. Laboratory evaluation of aqueous extract of botanicals

4.1.1.1. *Ovicidal effect of aqueous extracts on Tetranychus truncatus*

The botanicals were evaluated for ovicidal action against *T. truncatus* at three different concentrations *viz*., 5, 7.5 and 10 per cent. Data on the ovicidal effect of aqueous extract of the botanicals at different time intervals are presented in Table 2. The result showed that, hatchability was not significant among the treatments until 48 h after treatment.

After 72 h treatment, significantly higher hatchability of 98.67 per cent was recorded by 5 per cent extract of *Eucalyp*tus. This was followed by *Eucalyptus* (7.5 %), *Eucalyptus* (10%), *L. camara* (5%), *V. negundo* (5%), *O. sanctum* (5%) *V. negundo* (7.5%) and *L. camara* (7.5%) which showed per cent hatchability of 94.67, 93.33, 93.33, 92.00, 89.33 89.33 and 88.00, respectively and were on par with each

other. Significant hatchability was also recorded in 10 per cent *L. camara* (85.33%), 5 per cent *C. papaya* (85.33 %) and 7.5 per cent *C. papaya* (84.00%), which were on par with 5 per cent *B. monnieri* (78.67%), 10 per cent *C. papaya* (78.67%), 5 per cent *A. marmelos* (78.67%) and 7.5 per cent *B. monnieri* (76.00%). This was followed by *O. sanctum* (7.5 %), *A. marmelos* (7.5 %), *A. marmelos* (10%), *V. negundo* (10%) and *O. sanctum* (10%) which recorded hatchability of 70.67, 69.33, 69.33, 65.33 and 65.33 per cent.

By 96 h after treatment, 100 per cent hatchability was recorded in the treatments *Eucalyptus* (5, 7.5 and 10 %), *V. negundo* (5, 7.5 and 10%), *O. sanctu* (5 and 7.5%), *Q. indica* (5 and 7.5%), *B. monnieri* (5 and 7.5%) and *C. papaya* (5%), closely followed by *A. marmelos* (5%), *O. sanctum* (10%) and *Q. indica* (10%) all recording 98.67 per cent hatchability. The treatments, *L. camara* (5, 7.5 and 10%) with hatchability of 97.33, 96.00 and 96.00 per cent, respectively and *A. marmelos* (7.5 and 10%) with hatchability of 94.67 and 93.33 percent, respectively followed the above. Both *A. squamosa* (5%) and *B. monnieri* (10%) with hatchability of 89.33 per cent and *C. papaya* (10%) with 88.00 per cent hatchability were also found significant when compared to control. However, all treatments recorded 100 per cent hatchability by 120 h of treatment and hence none of the treatments were found significant.

Table 2. Effect of aqueous extract of botanicals on eggs of *Tetranychus truncatus*

Treatment	Hatchability (%)					Mortality at 120 h (%)
	24 h	48 h	72 h	96 h	120 h	
Lantana camera (5%)	0.00 (0.57)	0.00 (0.57)	93.33 [ab] (78.10)	97.33 [ab] (82.12)	100.00 (89.43)	0.00 (0.57)
Lantana camera (7.5%)	0.00 (0.57)	0.00 (0.57)	88.00 [ab] (73.09)	96.00 [ab] (82.86)	100.00 (89.43)	0.00 (0.57)
Lantana camera (10%)	0.00 (0.57)	0.00 (0.57)	85.33 [b] (67.52)	96.00 [ab] (80.49)	100.00 (89.43)	0.00 (0.57)
Aegle marmelos (5%)	0.00 (0.57)	0.00 (0.57)	78.67 [bc] (66.84)	98.67 [a] (85.77)	100.00 (89.43)	0.00 (0.57)
Aegle marmelos (7.5%)	0.00 (0.57)	0.00 (0.57)	69.33 [cd] (56.75)	94.67 [ab] (79.21)	100.00 (89.43)	0.00 (0.57)
Aegle marmelos (10%)	0.00 (0.57)	0.00 (0.57)	69.33 [cd] (56.64)	93.33 [ab] (77.58)	100.00 (89.43)	0.00 (0.57)
Eucalyptus spp. (5%)	0.00 (0.57)	0.00 (0.57)	98.67 [a] (85.77)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Eucalyptus spp. (7.5%)	0.00 (0.57)	0.00 (0.57)	94.67 [ab] (76.83)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Eucalyptus spp. (10%)	0.00 (0.57)	0.00 (0.57)	93.33 [ab] (77.58)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Vitex negundo (5%)	0.00 (0.57)	0.00 (0.57)	92.00 [ab] (73.92)	100.00 [a] (89.43)	100.00 (89.43	0.00 (0.57)
Vitex negundo (7.5%)	0.00 (0.57)	0.00 (0.57)	89.33 [ab] (71.82)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Vitex negundo (10%)	0.00 (0.57)	0.00 (0.57)	65.33 [cd] (54.24)	100.00 [a] (89.43)	100.000 (89.43)	0.00 (0.57)

Table 2. continued

Treatment	Hatchability (%)					Mortality at 120 h (%)
	24 h	48 h	72 h	96 h	120 h	
Ocimum sanctum (5%)	0.00 (0.57)	0.00 (0.57)	89.33 [ab] (71.19)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Ocimum sanctum (7.5%)	0.00 (0.57)	0.00 (0.57)	70.67 [c] (57.49)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Ocimum sanctum (10%)	0.00 (0.57)	0.00 (0.57)	65.33 [cd] (53.96)	98.67 [a] (85.77)	100.00 (89.43)	0.00 (0.57)
Quassia indica (5%)	0.00 (0.57)	0.00 (0.57)	50.67 [d] (45.36)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Quassia indica (7.5%)	0.00 (0.57)	0.00 (0.57)	14.67 [e] (22.37)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Quassia indica (10%)	0.00 (0.57)	0.00 (0.57)	8.00 [ef] (16.08)	98.67 [a] (85.77)	100.00 (89.43)	0.00 (0.57)
Bacopa monnieri (5%)	0.00 (0.57)	0.00 (0.57)	78.67 [bc] (62.51)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Bacopa monnieri (7.5%)	0.00 (0.57)	0.00 (0.57)	76.00 [bc] (60.81)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Bacopa monnieri (10%)	0.00 (0.57)	0.00 (0.57)	58.67 [d] (50.01)	89.33 [b] (71.54)	100.00 (89.43)	0.00 (0.57)
Carica papaya (5%)	0.00 (0.57)	8.00 (10.16)	85.33 [b] (67.81)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
Carica papaya (7.5%)	0.00 (0.57)	4.00 (7.14)	84.00 [b] (66.53)	94.67 [ab] (76.83)	100.00 (89.43)	0.00 (0.57)
Carica papaya (10%)	0.00 (0.57)	4.00 (7.14)	78.67 [bc] (62.51)	88.00 [b] (69.91)	100.00 (89.43)	0.00 (0.57)

Table 2. continued

Treatment	Hatchability (%)					Mortality at 120 h (%)
	24 h	48 h	72 h	96 h	120 h	
Annona squamosa (5%)	0.00 (0.57)	0.00 (0.57)	18.67 [e] (25.39)	89.33 [b] (74.19)	100.00 (89.43)	0.00 (0.57)
Annona squamosa (7.5%)	0.00 (0.57)	0.00 (0.57)	6.67 [ef] (11.89)	69.33 [c] (56.45)	100.00 (89.43)	0.00 (0.57)
Annona squamosa (10%)	0.00 (0.57)	0.00 (0.57)	0.00 [f] (0.57)	52.00 [d] (46.16)	100.00 (89.43)	0.00 (0.57)
Acorus calamus (5%)	0.00 (0.57)	0.00 (0.57)	0.00 [f] (0.57)	61.33 [cd] (51.68)	100.00 (89.43)	0.00 (0.57)
Acorus calamus (7.5%)	0.00 (0.57)	0.00 (0.57)	0.00 [f] (0.57)	62.67 [cd] (52.55)	100.00 (89.43)	0.00 (0.57)
Acorus calamus (10%)	0.00 (0.57)	0.00 (0.57)	0.00 [f] (0.57)	56.00 [d] (48.52)	100.00 (89.43)	0.00 (0.57)
Control	0.00 (0.57)	0.00 (0.57)	93.33 [ab] (77.58)	100.00 [a] (89.43)	100.00 (89.43)	0.00 (0.57)
CD (0.05)	**NS**	**NS**	**12.70**	**8.69**	**NS**	**NS**

Each value is mean of three replications
Figures in parentheses are arc sine transformed values
Means followed by common letter(s) do not significantly differ at P=0.05

4.1.1.2. *Adulticidal effect of aqueous extracts of botanicals on Tetranychus truncatus*

The adulticidal action of the botanicals was evaluated at three different concentrations viz., 5, 7.5 and 10 per cent. Data on adulticidal effect of aqueous botanical extracts on *T. truncatus*, at different time intervals are given in Table 3.

Among the treatments, 10 per cent *A. squamosa* (22.67 %) recorded significantly higher mortality after 24 h of treatment. This was followed by *A. squamosa* (7.5%), *O. sanctum* (10%), *A. squamosa* (5%), *B. monnieri* (10%), *O. sanctum* (7.5%) and *B. monnieri* (7.5%) recording per cent mortality of 17.33, 14.67, 12.00, 10.67, 8.00 and 6.67 respectively. *Q. indica* (10%), *B. monnieri* (5%) and *O. sanctum* (5%), each recording 4.00 per cent mortality and *Q. indica* (7.5%) with 2.67 per cent mortality were also found to be significant compared to control.

After 48 h of treatment, *A. squamosa* (10%) recorded significantly higher mortality of 61.33 per cent. *A. squamosa* (7.5 %) recorded a mortality of 44.00 per cent, which was on par with *L. camara* (10 %) recording 37.33 per cent mortality. This was followed by 5 per cent *A. squamosa* (32.00%), 10 per cent *B. monnieri* (29.33%), 10 per cent *C. papaya* (29.33%) and 7.5 per cent *B. mon*nieri (26.67 %) which were on par with *O. sanctum* (10%), *B. monnieri* (5%), *A. calamus* (10%) and *L. camara* (7.5%) that recorded per cent mortality of 25.33, 22.67, 21.33 and 21.33, respectively.

By 72 h of treatment, significantly higher mortality was observed in 10 and 7.5 per cent *A. squamosa* recording 76.00 and 69.33 per cent respectively, closely followed by 10 per cent extracts of *B. monnieri* (68.00%) and *C. papaya* (66.67%) as well as 5 per cent *A. squamosa* (62.67 %) recording mortality at par. *B. monnieri* (7.5%), *L. camara* (10 and 7.5%) and *B. monnieri* (5%) also showed significant mortality recording 53.33, 50.67, 44.00 and 41.33 per cent mortality, which were all at par. This was followed by 10 per cent *O. sanctum* (33.33%), which was on par with *L. camara* (5%), *Eucalyptus* (10 and 7.5%), *A. calamus* (10%), *C. papaya* (7.5%) and

V. negundo (10%) recording 32.00, 28.00, 26.67, 25.33, 24.00 and 18.67 per cent mortality.

At 96 h of treatment, *A. squamosa* (10 and 7.5%), *C. papaya* (10%), *A. squamosa* (5%) and *B. monnieri* (10%) were found to be on par with each other, recording per cent mortality of 94.67, 88.00, 86.67, 85.33 and 81.33, respectively. Mortality of 69.33 per cent was observed in 10 per cent extracts of both *A. calamus* and *L. camara*, which was on par with *B. monnieri* (7.5 and 5%) and *C. papaya* (7.5%) recording mortality of 62.67, 61.33 and 60.00 per cent, respectively. Significant mortality on par with each other was observed in 7.5 per cent *L. camara* (50.67 %), 5 per cent *L. camara* (41.33%), 10 per cent *Eucalyptus* (37.33%) and 10 per cent *O. sanctum* (37.33%). This was followed by *V. negundo* (10%), *Eucalyptus* (7.5%), *V. negundo* (7.5%), *Aegle marmelos* (10%) and *O. sanctum* (7.5%) recording mortality of 34.67, 33.33, 26.67, 24.00 and 22.67 per cent respectively.

After 120 h of treatment, highest mortality of 98.67 per cent was recorded by 10 per cent extracts of both *A. squamosa* and *C. papaya*, followed by *B. monnieri* (10%), *A. squamosa* (7.5%), *A. squamosa* (5%) recording mortality of 93.33, 92.00 and 88.00 per cent, respectively, on par with each other. The botanical extracts of 7.5 per cent *B. monnieri*, 10 per cent *A. calamus*, 7.5 per cent *C. papaya* , 10 per cent *L. camara* and 5 per cent *B. monnieri* recorded 78.67, 77.33, 74.67, 72.00 and 69.33 per cent mortality, respectively, all at par with each other. This was followed by *Eucalyptus* (10%), *L. camara* (7.5%), *V. negundo* (10%) and *L. camara* (5%) recording 52.00, 50.67, 45.33 and 41.33 per cent mortality, respectively. *V. negundo* (7.5%), *O. sanctum* (7.5%), *A. marmelos* (10%) and *C. papaya* (5%) recorded 37.33, 28.00, 25.33 and 25.33 per cent mortality, respectively. All other treatments were inferior to the above treatments, though recorded significantly higher mortality compared to control.

Table 3. Effect of aqueous extract of different botanicals on adults of *Tetranychus truncatus*

Treatment	Mortality (%)				
	24 h	48 h	72 h	96 h	120 h
Lantana camara (5%)	0.00 ^{f} (0.57)	14.67 ^{d} (22.37)	32.00 ^{cd} (33.96)	41.33 ^{cd} (39.78)	41.33 ^{cd} (39.78)
Lantana camara (7.5%)	0.00 (0.57)	21.33 ^{cd} (27.36)	44.00 ^{bc} (41.42)	50.67 ^{c} (45.36)	50.67 ^{c} (45.36)
Lantana camara (10%)	0.00 ^{f} (0.57)	37.33 ^{bc} (37.26)	50.67 ^{b} (45.45)	69.33 ^{b} (57.14)	72.00 ^{b} (59.256)
Aegle marmelos (5%)	0.00 ^{f} (0.57)	5.33 ^{de} (13.17)	6.67 ^{d} (14.79)	8.00 ^{e} (16.43)	9.33 ^{f} (17.71)
Aegle marmelos (7.5%)	0.00 ^{f} (0.57)	6.67 ^{de} (14.79)	9.33 ^{d} (17.71)	16.00 ^{e} (23.29)	21.33 ^{e} (27.36)
Aegle marmelos (10%)	0.00 ^{f} (0.57)	9.33 ^{de} (17.12)	17.33 ^{d} (24.16)	24.00 ^{de} (29.19)	25.33 ^{de} (30.12)
Eucalyptus sp. (5%)	0.00 ^{f} (0.57)	8.00 ^{de} (16.08)	12.00 ^{d} (20.09)	18.67 ^{e} (25.57)	22.67 ^{e} (28.19)
Eucalyptus sp. (7.5%)	0.00 ^{f} (0.57)	12.00 ^{de} (20.09)	26.67 ^{cd} (30.92)	33.33 ^{d} (35.15)	41.33 ^{cd} (39.96)
Eucalyptus sp. (10%)	0.00 ^{f} (0.57)	13.33 ^{de} (21.37)	28.00 ^{cd} (31.59)	37.33 ^{cd} (37.45)	52.00 ^{c} (46.16)
Vitex negundo (5%)	0.00 ^{f} (0.57)	2.67 ^{e} (5.86)	6.67 ^{d} (14.79)	16.00 ^{e} (22.48)	18.67 ^{ef} (24.98)
Vitex negundo (7.5%)	0.00 ^{f} (0.57)	10.67 ^{de} (18.81)	17.33 ^{d} (24.57)	26.67 ^{de} (31.04)	37.33 ^{d} (37.66)
Vitex negundo (10%)	0.00 ^{f} (0.57)	9.33 ^{de} (17.71)	18.67 ^{cd} (25.26)	34.67 ^{d} (36.04)	45.33 ^{cd} (42.29)

Table 3. continued

Treatment	Mortality (%)				
	24 h	**48 h**	**72 h**	**96 h**	**120 h**
Ocimum sanctum (5%)	4.00 ^{e} (9.51)	9.33 ^{de} (17.71)	9.33 ^{d} (17.71)	14.67 ^{e} (22.48)	14.67 ^{ef} (22.48)
Ocimum sanctum (7.5%)	8.00 ^{d} (16.08)	12.00 ^{de} (19.46)	16.00 ^{d} (23.47)	22.67 ^{de} (28.29)	28.00 ^{de} (31.91)
Ocimum sanctum (10%)	14.67 ^{bc} (22.47)	25.33 ^{cd} (30.12)	33.33 ^{c} (35.21)	37.33 ^{cd} (37.58)	41.33 ^{cd} (39.96)
Quassia indica (5%)	0.00 ^{f} (0.57)	4.00 ^{de} (9.51)	8.00 ^{d} (16.08)	9.33 ^{e} (17.36)	12.00 ^{ef} (20.09)
Quassia indica (7.5%)	2.67 ^{e} (7.88)	8.00 ^{de} (16.08)	10.67 ^{d} (18.46)	13.33 ^{e} (21.19)	17.33 ^{ef} (24.57)
Quassia indica (10%)	4.00 e (9.51)	10.67 ^{de} (18.18)	12.00 ^{d} (19.46)	14.67 ^{e} (22.37)	24.00 ^{e} (29.28)
Bacopa monnieri (5%)	4.00 ^{e} (9.51)	22.67 ^{cd} (28.02)	41.33 ^{bc} (39.95)	61.33 ^{bc} (51.68)	69.33 ^{b} (56.49)
Bacopa monnieri (7.5%)	6.67 ^{de} (14.79)	26.67 ^{c} (30.83)	53.33 ^{b} (46.88)	62.67 ^{bc} (52.48)	78.67 ^{b} (62.51)
Bacopa monnieri (10%)	10.67 ^{cd} (18.99)	29.33 ^{c} (32.42)	68.00 ^{ab} (55.78)	81.33 ^{ab} (65.82)	93.33 ^{a} (77.58)
Carica papaya (5%)	0.00 ^{f} (0.57)	2.67 ^{e} (5.86)	12.00 ^{d} (20.09)	18.67 ^{e} (25.26)	25.33 ^{de} (30.12)
Carica papaya (7.5%)	0.00 ^{f} (0.57)	5.33 ^{de} (10.79)	24.00 ^{cd} (28.79)	60.00 ^{bc} (50.78)	74.67 ^{b} (59.79)
Carica papaya (10%)	0.00 ^{f} (0.57)	29.33 ^{c} (32.74)	66.67 ^{ab} (54.89)	86.67 ^{a} (72.09)	98.67 ^{a} (85.77)

Table 3. continued

Treatment	Mortality (%)				
	24 h	**48 h**	**72 h**	**96 h**	**120 h**
Annona squamosa (5%)	12.00 [c] (20.09)	32.00 [c] (34.34)	62.67 [ab] (52.38)	85.33 [a] (67.99)	88.00 [ab] (70.19)
Annona squamosa (7.5%)	17.33 [b] (24.57)	44.00 [b] (41.52)	69.33 [a] (56.41)	88.00 [a] (69.91)	92.00 [a] (73.92)
Annona squamosa (10%)	22.67 [a] (28.29)	61.33 [a] (51.57)	76.00 [a] (60.72)	94.67 [a] (78.86)	98.67 [a] (85.77)
Acorus calamus (5%)	0.00 [f] (0.57)	4.00 [de] (9.51)	6.67 [d] (14.79)	14.67 [e] (22.19)	20.00 [ef] (26.49)
Acorus calamus (7.5%)	0.00 [f] (0.57)	8.00 [de] (16.43)	9.33 [d] (17.71)	13.33 [e] (21.09)	20.00 [ef] (26.49)
Acorus calamus (10%)	0.00 [f] (0.57)	21.33 [cd] (27.41)	25.33 [cd] (30.21)	69.33 [b] (56.41)	77.33 [b] (61.64)
Control	0.00 [f] (0.57)	0.00 (0.57)	2.67 [d] (7.88)	5.33 [e] (11.14)	6.67 [f] (14.79)
CD (0.05)	**3.32**	**11.06**	**14.72**	**14.66**	**12.15**

Each value is mean of three replications
Figures in parentheses are arc sine transformed values
Means followed by common letter(s) do not significantly differ at P=0.05

4.1.2. Laboratory evaluation of methanol extract of botanicals

The weight of the methanol extract of all the botanicals was recorded and per cent yield was calculated. The maximum yield of 7.14 per cent was recorded in seed extract of *A. squamosa*, followed by rhizome of *A. calamus* (5.82 %), leaves of *C. papaya* (4.82%) and *Q. indica* (4.56%), whole plant of *L. camara* (4.23%), leaves of *Eucalyptus* sp. (3.89%) and *A. marmelos* (3.68%), whole plant of *B. monnieri* (3.21%), leaves of *O. sanctum* (2.65%) and *V. negundo* (2.09%) (Table 4).

Table 4. Yield of methanolic extract of botanicals

Sl. No	Botanical	Yield (%)
1	*Lantana camara*	4.23
2	*Aegle marmelos*	3.68
3	*Eucalyptus* sp.	3.89
4	*Vitex negundo*	2.09
5	*Ocimum sanctum*	2.65
6	*Quassia indica*	4.56
7	*Bacopa monnieri*	3.21
8	*Carica papaya*	4.82
9	*Annona squamosa*	7.14
10	*Acorus calamus*	5.82

4.1.2.1. *Ovicidal effect of methanol extracts on Tetranychus truncatus*

The data on the ovicidal effect of methanol extract of the botanicals is furnished in Table 5. The results showed that the eggs did not hatch until 48 h in any of the treatments including control. After 72 h of treatment, highest hatchability of 85.33 per cent was recorded by *O. sanctum* (1 %), followed by *O. sanctum* (2%) and *Q. indica* (1%), each recording hatchability of 81.33 per cent, and all the three treatments were on par with each other. After 96 h of treatment, 100 per cent hatchability was recorded in the treatments *O. sanctum* (1 and 2%), *Eucalyptus* sp. (1 and 2%), *Q. indica* (1 and 2%), *C. papaya* (1%) and *V. negundo* (1%). However *V. negundo* (2%), *L. camara* (1%), *A. marmelos* (1%), *A. marmelos* (2%), and *L. camara* (2%) recorded hatchabilty of 97.33, 88.00, 86.67, 86.67, and 80.00 per cent, respectively. At 120 h, *L. camara* (2%), *V. negundo* (2%), *B. monnieri* (1% and 2%), *C. papaya* (2%), *A. squamosa* (1%) and *A. calamus* (1%) also recorded 100 per cent hatchabilty of eggs.

At 120 h, *Annona squamosa* (2%) and *Acorus calamus* (2%) recorded the highest egg mortality of 12 per cent, while *Aegle marmelos* (1 and 2 %) recorded 5.33 and 9.33 per cent mortality respectively. None of the other botanicals showed ovicidal activity (Table 5.).

Table 5. Effect of methanol extract of botanicals on eggs of *Tetranychus truncatus*

Treatment	Hatchability (%)					Mortality at 120 h (%)
	24 h	48 h	72 h	96 h	120 h	
Lantana camara (1%)	0.00 (0.57)	0.00 (0.57)	13.33 ^{hi} (17.83)	88.00 ^{bc} (69.91)	97.33 ^{a} (84.14)	2.67 ^{c} (5.86)
Lantana camara (2%)	0.00 (0.57)	0.00 (0.57)	2.67 ^{ij} (5.86)	80.00 ^{cd} (63.51)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Aegle marmelos (1%)	0.00 (0.57)	0.00 (0.57)	70.67 ^{bc} (57.56)	86.67 ^{c} (69.44)	94.67 ^{ab} (78.86)	5.33 ^{bc} (11.14)
Aegle marmelos (2%)	0.00 (0.57)	0.00 (0.57)	61.33 ^{cd} (51.66)	86.67 ^{c} (68.91)	90.67 ^{bc} (72.29)	9.33 ^{ab} (17.71)
Eucalyptus sp. (1%)	0.00 (0.57)	0.00 (0.57)	33.33 ^{g} (34.96)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Eucalyptus sp. (2%)	0.00 (0.57)	0.00 (0.57)	28.00 ^{g} (31.71)	100.00 ^{a} (89.43)	100.00 ^{a} (89.427)	0.000 ^{c} (0.573)
Vitex negundo (1%)	0.00 (0.57)	0.00 (0.57)	56.00 ^{de} (48.48)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Vitex negundo (2%)	0.00 (0.57)	0.00 (0.57)	46.67 ^{ef} (43.08)	97.33 ^{ab} (84.14)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Ocimum sanctum (1%)	0.00 (0.57)	0.00 (0.57)	85.33 ^{a} (67.81)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Ocimum sanctum (2%)	0.00 (0.57)	0.00 (0.57)	81.33 ^{ab} (64.61)	100.00 ^{a} (89.43)	100.00^{a} (89.43)	0.00 ^{c} (0.57)
Quassia indica (1%)	0.00 (0.57)	0.00 (0.57)	81.33 ^{ab} (64.43)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Quassia indica (2%)	0.00 (0.57)	0.00 (0.57)	73.33 ^{abc} (58.96)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)

Table 5. continued

Treatment	Hatchability (%)					Mortality at 120 h (%)
	24 h	48 h	72 h	96 h	120 h	
Bacopa monnieri (1%)	0.00 (0.57)	0.00 (0.57)	34.67 ^{fg} (36.02)	68.00 ^{e} (55.99)	100.00 ^{a} (89.43)	0.000 ^{c} (0.573)
Bacopa monnieri (2%)	0.00 (0.57)	0.00 (0.57)	25.33 ^{gh} (30.12)	44.000 ^{g} (41.523)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Carica papaya (1%)	0.00 (0.57)	0.00 (0.57)	61.33 ^{cd} (51.65)	100.00^{a} (89.427)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Carica papaya (2%)	0.00 (0.57)	0.00 (0.57)	32.00 ^{g} (34.34)	54.667 ^{f} (47.74)	100.00 ^{a} (89.43))	0.00 ^{c} (0.57)
Annona squamosa (1%)	0.00 (0.57)	0.00 (0.57)	24.00 ^{gh} (29.12)	74.67 ^{de} (60.01)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Annona squamosa (2%)	0.00 (0.57)	0.00 (0.57)	0.00 ^{j} (0.57)	22.67 ^{h} (28.29)	88.000 ^{c} (73.095)	12.000 ^{a} (16.905)
Acorus calamus (1%)	0.00 (0.57)	0.000 (0.57)	24.00 ^{gh} (64.73)	74.67 ^{de} (60.01)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
Acorus calamus (2%)	0.00 (0.57)	0.00 (0.57)	0.00 ^{j} (0.57)	22.667 ^{h} (28.29)	88.000 ^{c} (73.09)	12.00 ^{a} (16.90)
Control	0.00 (0.57)	0.00 (0.57)	84.00 ^{a} (66.53)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)	0.00 ^{c} (0.57)
CD (0.05)	**NS**	**NS**	**12.91**	**10.44**	**5.93**	**5.93**

Each value is mean of three replications
Figures in parentheses are arc sine transformed values
Means followed by common letter(s) do not significantly differ at P=0.05

4.1.2.2. *Adulticidal effect of methanol extracts of botanicals on Tetranychus truncatus*

Data on the adulticidal activity of methanol extracts of different botanicals on *T. truncatus* at different time intervals is presented in Table 6.

After 24 h of treatment, *A. calamus* (2%) recorded significantly higher mortality of 90.67 per cent followed *A. calamus* (1%) recording 72.00 per cent mortality. *C. papaya* (2%), *A. marmelos* (2%), *C. papaya* (1%) and *A. marmelos* (1%) recorded 36.00, 16.00, 9.33 and 4.00 per cent mortality, respectively. By 48 h of treatment, *A. calamus* (2%) recorded 100 per cent mortality, closely followed by two per cent extract of *C. papaya* (96.00%) and one per cent extract *A. calamus* (92.00 %) which were on par with each other. This was followed by one per cent extract of *C. papaya* also recorded significant mortality (70.67 %). *A. squamosa* (2%), *A. squamosa* (1%), *B. monnieri* (2%) and *A. marmelos* (2%) recorded 22.67, 20.00, 16.00 and 16.00 per cent mortality of adult mite, respectively and were on par with each other. This was followed by *O. sanctum* (2%), *B. monnieri* (1%) and *Q. indica* (2%), which recorded mortality of 12.00, 10.67 and 9.33 per cent, respectively. The treatments *viz.*, *A. marmelos* (1%), *V. negundo* (2%) and *O. sanctum* (1%), each recorded 4.00 per cent mortality, while *L. camara* (1%) and *L. camara* (2% recorded 2.67 and 1.33 per cent mortality, respectively.

After 72 h of treatment, 100 per cent mortality was also recorded by *A. calamus* (1 and 2%) and *C. papaya* (2%). Mortality of *A. marmelos* (2%) and *C. papaya* (1%) were found to be on par, recording 86.67 and 82.67 per cent respectively, while *A. squamosa* (2%) recorded 41.33 per cent mortality. One per cent *A. squamosa* (34.67 %) and two per cent *B. monnieri* (30.667 %) also recorded significant mortality, on par with *A. squamosa* (2%). This was followed by *A. marmelos* (1%), *O. sanctum* (2%) and *B. monnieri* (1%) which recorded 28.00, 26.67, and 25.33 per cent mortality on par with each other. *Q. indica* (2%), *Eucalyptus* (2%), *L. camara* (2%), *Eucalyptus* (1%), *V. negundo* (1%), *O. sanctum* (1%) recorded 20.00, 14.67, 10.67, 9.33, 8.00 and 6.67 per cent mortality, respectively. Both *L.*

camara and *Q. indica* at one per cent concentration recorded mortality of 5.33 per cent only.

By 96 h of application of treatment, *C. papaya* (1%) also recorded 100 per cent mortality of adult mite which was on par with two per cent extract of *A. marmelos* (93.33%), followed by *A. squamosa* (2%) *A. marmelos* (1%), *A. squamosa* (1%) and *B. monnieri* (2%) which were on par with each other recording mortality of 52.00, 49.33, 48.00 and 41.33 per cent respectively. This was followed by *O. sanctum* (2%) and *B. monnieri* (1%), each recording per cent mortality of 38.67, and *Q. indica* (2%) recording 28.00 per cent. *V. negundo* (2%), *V. negundo* (1%), *Eucalyptus* (2%), *O. sanctum* (1%), *Eucalyptus* (1%) recorded mortality of 24.00, 22.67, 21.33, 17.33 and 16.00 per cent, respectively, on par with each other. *Lantana camara* (2%), *Q. indica* (1%) and *L. camara* (1%) recorded 14.67, 10.67 and 6.67 per cent mortality, respectively.

On 120 h of treatment, *A. marmelos* (2%) recorded significantly higher mortality of 98.67 per cent of gravid females of *T. truncatus*. This was followed by two per cent *A. squamosa* (70.67 %), two per cent *B. monnieri* (62.667%) and one per cent *A. marmelos* (61.33 %), which were on par with each other. The mortality recorded by one per cent concentrations of *A. squamosa* (56.00%) and *B. monnieri* (54.67%) were on par with each other, followed by two per cent *O. sanctum* (50.67%). *Q. indica* (2%), *V. negundo* (2%), *V. negundo* (1%), *Eucalyptus* (2%), *O. sanctum* (1%), *L. camara* (2%), *Eucalyptus* sp. (1%) *Q. indica* (1%) and *L. camara* (1%) recorded 40.00, 38.67, 30.67, 30.67, 21.33, 20.00, 18.67, 13.33 and 9.33 per cent mortality of adult mite.

Table 6. Effect of methanol extract of botanicals on gravid females of *Tetranychus truncatus*

Treatment	Mortality (%)				
	24 h	**48 h**	**72 h**	**96 h**	**120 h**
Lantana camara (1%)	0.00^{f} (0.57)	2.67 ^{ghi} (5.86)	5.33 ^{hi} (13.17)	6.67 ^{hi} (14.45)	9.33 ^{ij} (17.18)
Lantana camara (2%)	0.00^{f} (0.57)	1.33 ^{hi} (4.23)	10.67 ^{ghi} (18.18)	14.67 ^{fghi} (22.19)	20.00 ^{ghi} (26.09)
Aegle marmelos (1%)	4.00^{ef} (9.51)	4.00 ^{fghi} (9.51)	28.00 ^{de} (31.59)	49.33 ^{bc} (44.55)	61.33 ^{bcd} (51.68)
Aegle marmelos (2%)	16.00^{d} (23.47)	16.00 ^{cde} (23.47)	86.67 ^{b} (73.67)	93.33 ^{a} (80.76)	98.67 ^{a} (85.77)
Eucalyptus sp. (1%)	0.00^{f} (0.57)	0.00 ^{i} (0.57)	9.33 ^{ghi} (17.18)	16.00^{efgh} (23.11)	18.67 ^{hi} (25.38)
Eucalyptus sp. (2%)	0.00^{f} (0.57)	0.00 ^{i} (0.57)	14.67 ^{fgh} (22.47)	21.33 ^{efg} (27.36)	30.67 ^{fg} (33.59)
Vitex negundo (1%)	0.00^{f} (0.57)	1.33^{hi} (4.23)	8.00 ^{hi} (16.08)	22.67 ^{efg} (28.19)	30.67 ^{fg} (33.50)
Vitex negundo (2%)	0.00^{f} (0.57)	4.00 ^{fghi} (9.51)	9.33 ^{ghi} (17.18)	24.00^{ef} (29.28)	38.67 ^{f} (38.34)
Ocimum sanctum (1%)	0.00^{f} (0.57)	4.00 ^{fghi} (9.51)	6.67 ^{hi} (14.79)	17.33 ^{efgh} (24.39)	21.33 ^{gh} (27.36)
Ocimum sanctum (2%)	0.00^{f} (0.57)	12.00 ^{def} (20.09)	26.67 ^{de} (31.04)	38.67 ^{cd} (38.25)	50.67 ^{de} (45.37)
Quassia indica (1%)	0.00^{f} (0.57)	0.00 ^{i} (0.57)	5.33 ^{hi} (13.17)	10.67 ^{ghi} (18.81)	13.333 ^{hij} (21.19)
Quassia indica (2%)	0.00^{f} (0.57)	9.33 ^{efgh} (11.03)	20.00 ^{efg} (26.09)	28.00 ^{de} (31.71)	40.00 ^{ef} (39.18)

Table 6. continued

Treatment	Mortality (%)				
	24 h	**48 h**	**72 h**	**96 h**	**120 h**
Bacopa monnieri (1%)	0.00^{f} (0.57)	10.667 ^{efg} (18.81)	25.33 ^{def} (30.12)	38.67 ^{cd} (38.39)	54.67 ^{cd} (47.71)
Bacopa monnieri (2%)	2.67^{f} (5.86)	16.00^{cde} (23.47)	30.67 ^{cde} (33.55)	41.33 ^{bc} (39.98)	62.67 ^{bc} (52.44)
Carica papaya (1%)	9.33^{e} (14.81)	70.67^{b} (57.37)	82.67 ^{b} (65.89)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)
Carica papaya (2%)	36.00^{c} (36.85)	96.00^{a} (82.86)	100.00^{a} (89.43)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)
Annona squamosa (1%)	0.00^{f} (0.57)	20.00^{cd} (26.49)	34.67^{cd} (36.04)	48.00^{bc} (43.84)	56.00 ^{cd} (48.45)
Annona squamosa (2%)	0.00^{f} (0.57)	22.67^{c} (28.36)	41.33 ^{c} (39.98)	52.00 ^{b} (46.15)	70.67 ^{b} (57.28)
Acorus calamus (1%)	72.00^{b} (58.29)	92.00^{a} (76.47)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)
Acorus calamus (2%)	90.67 ^{a} (72.82)	100.00^{a} (89.43)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)	100.00 ^{a} (89.43)
Control	0.00^{f} (0.57)	0.00 ^{i} (0.57)	1.33 ^{i} (4.23)	5.33 ^{i} (13.17)	5.33 ^{j} (13.17)
CD (0.05)	**5.95**	**8.80**	**11.40**	**12.96**	**11.19**

Each value is mean of three replications
Figures in parentheses are arc sine transformed values
Means followed by common letter(s) do not significantly differ at P=0.05

4.2. IDENTIFICATION OF BIOACTIVE SOLVENT FRACTION OF PROMISING PLANT EXTRACTS

Based on the results of bioassay studies conducted in the laboratory, *Acorus calamus* was selected for bioassay guided fractionation, to identify the bioactive solvent fraction in it.

4.2.1. Laboratory evaluation of solvent fraction by liquid liquid partitioning

Bioassay guided fractionation of *A. calamus* was carried out using three different solvents *viz.,* hexane, chloroform and methanol sequentially against egg and adult of *T. truncatus* separately. The solvents methanol, hexane and chloroform recorded yield of 4.48, 3.91 and 3.48 per cent, respectively (Table 7).

Table 7. Yield of solvent fractions of *Acorus calamus*

Sl. No	***Acorus calamus* solvent fraction**	**Yield (%)**
1	Hexane fraction	3.91
2	Chloroform fraction	3.48
3	Methanol fraction	4.48

4.2.1.1. *Ovicidal effect of Acorus calamus solvent fraction on Tetranychus truncatus*

The ovicidal effect of hexane, chloroform and methanol fraction of *A. calamus* was evaluated against *T. truncatus* at three different concentrations viz., 0.3, 0.5 and 0.7 per cent. Data on the ovicidal activity at different time intervals is presented in Table 8.

The eggs did not hatch until 48 h, in any of the treatments tested. However, after 72 h after treatment, significantly higher hatchability was observed in 0.3 per cent chloroform fraction (92 %), followed by 0.3 per cent hexane (84 %). The treatments, 0.5 per cent chloroform fraction (81.33%), 0.5 per cent hexane (77.33 %) and 0.3 per cent methanol fraction (77.33 %), all of which were on par with each other. Both hexane and chloroform fraction at 0.7 per cent concentration recorded 73.33 per cent hatchability. The treatment, 0.5 per cent methanol fraction recorded 53.33 per cent hatchability whereas, no hatchability was recorded in 0.7 per cent methanol fraction. The hexane and chloroform control recorded cent per cent hatchability, whereas the methanol control recorded 89.33 per cent hatchability.

By 96 h after treatment, 100 per cent hatchability was observed in all the three concentrations of hexane fraction as well as in 0.3 per cent methanol fraction. Chloroform fraction (0.3%) also showed hatchability (97.33 %) on par with the above treatments. This was followed by 0.5 per cent chloroform fraction, 0.7 per cent chloroform fraction and 0.5 per cent methanol fraction recording hatchability of 93.33, 84.00 and 65.33 per cent, respectively which differed significantly from one another. No hatchability was recorded in 0.7 per cent methanol fraction. The control treatments recorded cent per cent hatchability.

After 120 h of treatment, all treatments except methanol fraction at concentrations 0.5 and 0.7 per cent recorded 100 per cent hatchability. While 70.67 per cent of the eggs hatched in 0.5 per cent methanol fraction, none of the eggs hatched in 0.7 per cent methanol fraction after fifth day of treatment. Observation on hatchability of eggs was continued in the treatment, 0.7 percent methanol fraction.

However, no hatching was observed further, and by 7th day, the eggs were all found shrunken and discolored confirming mortality (Plate 19).

Mortality of egg after 120 h of treatment was calculated based on per cent hatchability. It was found that methanol fraction at 0.7 per cent concentration resulted in 100 per cent mortality, followed by 0.5 per cent methanol fraction recording 29.33 per cent mortality. None of the other fractions showed ovicidal effect.

Table 8. Effect of *Acorus calamus* solvent fractions on eggs of *Tetranychus truncatus*

Treatment	Hatchability (%)					Mortality at 120h (%)
	24 h	48 h	72 h	96 h	120 h	
Hexane 0.3%	0.00 (0.57)	0.00 (0.57)	84.00 [cd] (66.53)	100.00 [a] (89.43	100.00 [a] (89.43)	0.00 [c] (0.57)
Hexane 0.5%	0.00 (0.57)	0.00 (0.57)	77.33 [de] (61.64)	100.00 [a] (89.43)	100.00 [a] (89.43)	0.00 [c] (0.57)
Hexane 0.7%	0.00 (0.57)	0.00 (0.57)	73.33[e] (59.01)	100.00 [a] (89.43)	100.00 [a] (89.43)	0.00 [c] (0.57)
Chloroform fraction 0.3%	0.00 (0.57)	0.00 (0.57)	92.00 [b] (73.92)	97.33 [ab] (82.12)	100.00 [a] (89.43)	0.00 [c] (0.57)
Chloroform fraction 0.5%	0.00 (0.57)	0.00 (0.57)	81.33 [d] (64.43)	93.33 [b] (75.20)	100.00 [a] (89.43)	0.00 [c] (0.57)
Chloroform fraction 0.7%	0.00 (0.57)	0.00 (0.57)	73.33 [e] (59.01)	84.00 [c] (66.53)	100.00 [a] (89.43)	0.00 [c] (0.57)
Methanol fraction 0.3%	0.00 (0.57)	0.00 (0.57)	77.33 [de] (59.01)	100.00 [a] (89.43)	100.00 [a] (89.43)	0.00 [c] (0.57)
Methanol fraction 0.5%	0.00 (0.57)	0.00 (0.57)	53.33 [f] (61.59)	65.33 [d] (54.02)	70.67 [b] (57.37)	29.33 [b] (32.63)

Table 8. continued

Treatment	Hatchability (%)					Mortality at 120h (%)
	24 h	48 h	72 h	96 h	120 h	
Methanol fraction 0.7%	0.00 (0.57)	0.00 (0.57)	0.00 [g] (0.57)	0.00 [e] (0.57)	0.00 [c] (0.57)	100.00 [a] (89.43)
Control (Hexane)	0.00 (0.57)	0.00 (0.57)	100.00 [a] (89.43)	100.00 [a] (89.43)	100.00[a] (89.43)	0.00 [c] (0.57)
Control (Chloroform)	0.00 (0.57)	0.00 (0.57)	100.00 [a] (89.43)	100.00 [a] (89.43)	100.00[a] (89.43)	0.00 [c] (0.57)
Control (Methane)	0.00 (0.57)	0.00 (0.57)	89.33[bc] (71.19)	100.00 [a] (89.43)	100.00 [a] (89.43)	0.00 [c] (0.57)
CD(0.05)	**NS**	**NS**	**5.35**	**3.10**	**2.57**	**2.57**

Each value is mean of three replications
Figures in parentheses are arc sine transformed values
Means followed by common letter(s) do not significantly differ by LSD at P=0.05%

4.2.1.2. *Adulticidal effect of Acorus calamus solvent fractions on Tetranychus truncatus*

The adulticidal effect of hexane, chloroform and methanol fraction of *Acorus calamus* was evaluated against *T. truncatus* at three different concentrations viz., 0.3, 0.5 and 0.7 per cent. Data on the adulticidal activity at different time intervals is presented in Table 9.

Within 24 h of treatment, methanol fraction of *A. calamus* (0.5 and 0.7 %) recorded 100 per cent mortality of adult mites (Plate 20). *Acorus calamus* at 0.3 per cent also showed significantly higher mortality (98.67%), on par with the higher

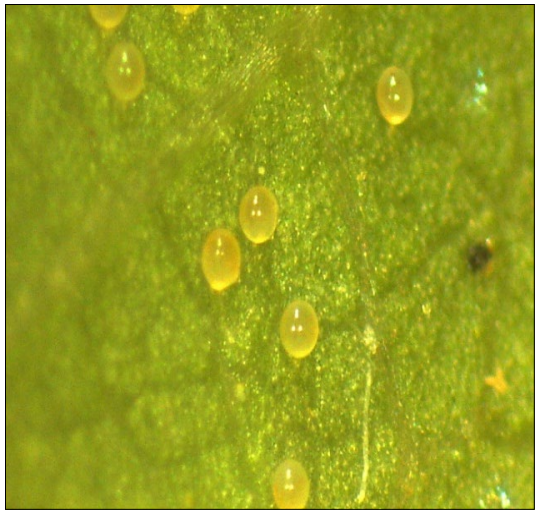

Plate 19a. Live eggs

Plate 19b. Dead eggs

Plate 19. Effect of 0.7 % methanol fraction of *Acorus calamus* on eggs of *Tetranychus truncatus*

Plate 20a. Live adults

Plate 20b. Dead adults

Plate 20. Effect of 0.7 % methanol fraction of *Acorus calamus* on adults of *Tetranychus truncatus*

concentrations. This was followed by chloroform fraction (0.7 %) recording 58.67 per cent mortality. Chloroform fraction (0.5%) and hexane fraction (0.3%) recorded 33.33 and 32.00 per cent mortality, respectively. However, chloroform fraction (0.3%) and hexane fraction (0.3 and 0.5%) recorded significantly lower mortality of 21.33 and 14.67 per cent, respectively.

After 48 h of treatment, 100 per cent mortality was also recorded by 0.3 per cent methanol fraction and 0.7 per cent chloroform fraction, closely followed by 0.7 per cent hexane (98.67 %), which were on par with each other. Hexane fraction (0.5 %), hexane fraction (0.3 %) and chloroform fraction (0.5%) recorded 68.00, 66.67 and 65.33 per cent mortality respectively, and were on par. Significantly lower mortality of 37.33 per cent was recorded by chloroform (0.3%). However, no mortality was observed in control treatments.

After 72 h of treatment, 100 per cent mortality was also recorded by 0.7 per cent hexane and 0.5 per cent chloroform fraction, closely followed by 0.5 per cent hexane, with 93.33 per cent mortality, which were on par with each other. This was followed by chloroform fraction (0.3%) and hexane fraction (0.3%), recording mortality of 85.33 and 81.33 per cent, respectively. The chloroform control recorded mortality of 5.33 per cent, whereas the hexane and methanol control recorded 4.00 per cent mortality of adult mites.

By 96 h of treatment, all concentrations of different solvent extracts recorded 100 per cent mortality. However, both hexane and methanol control treatments recorded only 6.67 per cent mortality and the chloroform control recorded 8.00 per cent mortality of adult *T. truncatus*.

Table 9. Effect of *Acorus calamus* solvent fractions on adults of *Tetranychus truncatus*

Treatment	Mortality (%)			
	24 h	48 h	72 h	96 h
Hexane 0.3%	14.67 [d] (22.37)	66.67 [b] (55.00)	81.33 [b] 65.019	100.00 [a] (89.43)
Hexane 0.5%	14.67 [d] (22.47)	68.00 [b] (55.78)	93.33 [a] 75.553	100.00 [a] (89.43)
Hexane 0.7%	32.00 [c] (34.34)	98.67 [a] (85.77)	100.00 [a] 89.43	100.00 [a] (89.43)
Chloroform fraction 0.3%	21.33 [d] (27.49)	37.33 [c] (37.67)	85.33 [b] (68.44)	100.00 [a] (89.43)
Chloroform fraction 0.5%	33.33[c] (35.21)	65.33 [b] (53.98)	100.00 [a] (89.43)	100.00 [a] (89.43)
Chloroform fraction 0.7%	58.67 [b] (50.13)	100.00 [a] (89.43)	100.00 [a] (89.43)	100.00 [a] (89.43)
Methanol fraction 0.3%	98.67 [a] (85.77)	100.00 [a] (89.43)	100.00 [a] (89.43)	100.00 [a] (89.43)
Methanol fraction 0.5%	100.00 [a] (89.43)	100.00 [a] 89.43)	100.00 [a] (89.43)	100.00 [a] (89.43)
Methanol fraction 0.7%	100.00 [a] (89.43)	100.00 [a] (89.43)	100.00 [a] (89.43)	100.00 [a] (89.43)

Table 9. continued

Treatment	Mortality (%)			
	24 h	**48 h**	**72 h**	**96 h**
Control (Hexane)	0.00 [e] (0.57)	0.00 [d] (0.57)	4.00 [c] (9.51)	6.67 [b] (14.79)
Control (Chloroform)	0.00 [e] (0.57)	0.00 [d] (0.57)	5.33 [c] (13.17)	8.00 [b] (16.43)
Control (Methanol)	0.00 [e] (0.57)	0.00 [d] (0.57)	4.00 [c] (9.51)	6.67 [b] (14.79)
CD(0.05)	**13.96**	**8.62**	**6.50**	**1.55**

Each value is mean of three replications
Figures in parentheses are arc sine transformed values
Means followed by common letter(s) do not significantly differ at P=0.05

4.2.2. Evaluation of botanical extracts against *Tetranychus truncatus* in polyhouse

An experiment was conducted to evaluate the efficacy of botanical extracts viz., methanol fraction of *A. calamus* (0.3 and 0.5%) and aqueous extract of *A. squamosa* (7.5%) along with neem oil emulsion (2%), HMO (2.5%) and spiromesifen (100 g a.i/ ha) against *T. truncatus* on cucumber plant in polyhouse. The mean number of mites before application of treatments ranged from 18.90 to 22.62 per cm^2 of leaf area (Table 10).

First day after treatment, the lowest mean number of mites was recorded by methanol fraction of *A. calamus* 0.5% (6.48 per cm^2) followed by spiromesifen (8.78 per cm^2). The treatments, methanol fraction of *A. calamus* 0.3% and HMO 2.5% also

showed significant reduction in mite population recording 11.72 and 14.19 mites per cm^2 leaf area, respectively. The number of mites in the treatments, *A. squamosa* extract (17.26 per cm^2) and neem oil (17.37 per cm^2) emulsion were on par. However, 23.29 mites per cm^2 leaf area were recorded in the control.

Third day after spraying, the mite population was significantly lower in the acaricide, spiromesifen (0.94 per cm^2), followed by *A. calamus* 0.5% (3.54 per cm^2 leaf) and *A. calamus* 0.3% (4.06 per cm^2), which were on par with each other. This was followed by *A. squamosa* extract (9.24 per cm^2), HMO (9.85 per cm^2) and neem oil emulsion (12.52 per cm^2). The control treatment recorded a mite population of 23.86 per cm^2.

At seven days of spraying, no mites were recorded on the plants treated with spiromesifen, while the treatments 0.5 and 0.3 per cent extract of *A. calamus* recorded 0.56 and 2.32 mites per cm^2 leaf area, respectively. The treatments, *A. squamosa*, HMO and neem oil emulsion also recorded significantly lower mite population of 5.96, 7.20 and 8.40 mites per cm^2, respectively compared to control (26.78 per cm^2).

After seven days of treatment application, 100 per cent reduction in mite population was observed in the treatment spiromesifen. This was followed by *A. calamus* (0.5%), *A. calamus* (0.3%), *A. squamosa* (7.5%), HMO (2.5 %) and neem oil emulsion (2%) recording population reduction of 97.04, .89.68, 71.18, 66.91 and 61.25 per cent, respectively (Table 10).

By ten days of treatment application, the mean number of mites in plants treated with 0.5 per cent extract of *A. calamus* was also found to be zero, which was on par with 0.3 per cent extract of *A. calamus* recording 0.20 mites per cm^2 leaf area. This was followed by *A. squamosa* extract (2.59 per cm^2) and HMO (2.45 per cm^2) which were on par with each other. Neem oil emulsion (4.28 per cm^2) also recorded significantly lower mite population compared to control treatment (25.45 per cm^2).

After fourteen days of spraying, the treatments methanol fraction extract of *A. calamus* (0.3 %) and horticultural mineral oil (2.5%) also recorded mite population of zero. Aqueous extract of *A. squamosa* and neem oil emulsion also recorded significantly lower mite population of 0.66 and 1.24 mites per cm^2 leaf area, respectively compared to control treatment (24.39 mites per cm^2).

Fourteen days post treatment, all treatments except 7.5 per cent *A. squamosa* (98.81%) and 2 per cent neem oil emulsion (94.28%) recorded 100 per cent reduction in mite population (Table10).

Table 10. Effect of treatments on *Tetranychus truncatus* on cucumber in polyhouse

Sl. No	Treatment	Precount	Mean no of mites/ cm^2 leaf area			Reduction in mite count 7 DAT (%)	Mean no of mites/ cm^2 leaf area		Reduction in mite count 14 DAT (%)
			1 DAT	3 DAT	7 DAT		10 DAT	14DAT	
1	*Acorus calamus* (0.3%)	22.48 (4.74)	11.72 [d] (3.42)	4.06 [e] (2.01)	2.32 [e] (1.68)	89.68	0.20 [d] (0.84)	0.00 [d] (0.71)	100
2	*Acorus calamus* (0.5%)	18.90 (4.35)	6.48 [f] (2.54)	3.54 [e] (1.88)	0.56 [f] (1.03)	97.04	0.00 [d] (0.71)	0.00 [d] (0.71)	100
3	*Annona squamosa* (7.5%)	20.68 (4.55)	17.26 [b] (4.15)	9.24 [d] (3.04)	5.96 [d] (2.54)	71.18	2.59 [c] (1.76)	0.66 [c] (1.76)	98.81
4	Neem oil emulsion (2%)	21.68 (4.66)	17.37 [b] (4.17)	12.52 [b] (3.54)	8.40 [b] (2.98)	61.25	4.28 [b] (2.18)	1.24 [b] (2.18)	94.28
5	HMO (2.5%)	21.76 (4.66)	14.19 [c] (3.76)	9.85 [c] (3.14)	7.20 [c] (2.77)	66.91	2.45 [c] (1.71)	0.00 [d] (0.71)	100
6	Spiromesifen (100 g a.i/ ha)	22.62 (4.76)	8.78 [e] (2.96)	0.94 [f] (0.97)	0.00 [g] (0.71)	100	0.00 [d] (0.71)	0.00 [d] (0.71)	100
7	Control	22.49 (4.74)	23.29 [a] (4.83)	23.86 [a] (4.88)	26.78 [a] (5.22)	-	25.45 [a] (5.09)	24.39 [a] (5.09)	-

Figures in parentheses are arc sine transformed values
Means followed by common letter(s) do not significantly differ at P=0.05%

4.3. EVALUATION OF SAFETY OF BOTANICAL FRACTION AGAINST THE PREDATORY MITE, *NEOSIEULUS LONGISPINOSUS*

Safety of methanol fraction of *A. calamus* was evaluated in the laboratory on eggs and adults of the predatory mite, *N. longispinosus*, along with 2 per cent neem oil emulsion and 2.5 per cent HMO.

4.3.1. Ovicidal effect of methanol fraction of *Acorus calamus* on *Neosieulus longispinosus*

The effect of methanol fraction of *A. calamus* on egg of *N. longispinosus* was studied at three different concentrations of 0.3, 0.5 and 0.7 per cent. Data on the ovicidal activity at different time intervals is presented in Table 11.

After 24 h of treatment, HMO (2.5%) showed significantly higher hatchability of 16.67 per cent, followed by neem oil emulsion (2%) with 11.11 per cent hatchability. However, none of the eggs treated with methanol fractions hatched.

By 48 h of treatment, significant hatchability of 33.33 per cent was recorded in HMO (2.5%), followed by neem oil emulsion (2%) and methanol fraction (0.3%) recording hatchability of 27.78 and 22.22 per cent respectively, all of which were on par with each other. The treatment, methanol fraction (0.5%) recorded hatchability of 11.11 per cent, while no eggs hatched in the treatment, methanol fraction (0.7%).

On 72 h of treatment, 100 per cent hatchability was recorded both in 0.3 and 0.5 per cent methanol fraction. Horticultural mineral oil (2.5%) recorded hatchability of 94.44 per cent, on par with the above treatments. Significantly higher hatchability was also observed in neem oil emulsion (2%) recording 88.89 per cent. However, at the highest concentration of methanol fraction (0.7%) tested, hatchability was nil even after 72 h of treatment.

Based on the hatchability of eggs, mortality of egg after 72 h of treatment was calculated. Methanol fraction (0.7%) showed 100 per cent egg mortality (Plate 21), followed by neem oil emulsion (2%) and HMO (2.5%) recording 11.11 and 5.56 per cent egg mortality respectively. No mortality of eggs was recorded in 0.3 and 0.5 per cent methanol fraction.

Table 11. Effect of methanol fraction of *Acorus calamus* on egg of *Neosieulus longispinosus*

Treatment	Hatchability (%)			Mortality at 72 h (%)
	24 h	48 h	72 h	
Methanol fraction (0.3%)	0.00 [b] (1.17)	22.22 [bc] (27.81)	100.00 [a] (88.83)	0.00 [c] (1.17)
Methanol fraction (0.5%)	0.00 [b] (1.17)	11.11 [cd] (16.46)	100.00 [a] (88.83)	0.00 [c] (1.17)
Methanol fraction (0.7%)	0.00 [b] (1.17)	0.00 [d] (1.17)	0.000 [c] (1.17)	100.00 [a] (88.83)
Neem oil emulsion (2%)	11.11 [b] (16.46)	27.78 [bc] (31.54)	88.89 [b] (73.54)	11.11 [b] (16.46)
HMO (2.5%)	16.67 [ab] (1.17)	33.33 [b] (34.79)	94.44 [ab] (81.19)	5.56 [bc] (8.81)
Control	33.33 [a] (20.18)	100.00 [a] (88.83)	100.00 [a] (88.83)	0.00 [c] (1.17)
CD (0.05%)	**18.49**	**17.11**	**9.89**	**9.89**

Each value is mean of three replications
Figures in parentheses are arc sine transformed values
Means followed by common letter(s) do not significantly differ at P=0.05%

4.3.2. Adulticidal effect of methanol fraction of *Acorus calamus* on *Neosieulus longispinosus*

Data on the adulticidal effect of methanol fraction of *A. calamus* on *N. longispinosus* at different concentrations and time intervals is presented in Table 12.

On 24 h after treatment, 0.7 per cent methanol fraction recorded 100 per cent mortality (Plate 22), followed by 0.5 and 0.3 per cent methanol fraction recording mortality of 77.78 and 38.89 per cent, respectively. However, the treatments, 2 per cent neem oil emulsion (11.11 %) and 2.5 per cent HMO (5.56 %) recorded mortality on par with control (0.00 %).

After 48 h of treatment, 0.5 per cent methanol fraction also recorded 100 per cent mortality, closely followed by 0.3 per cent methanol fraction showing 94.44 per cent mortality and was on par with each other. Neem oil emulsion (2%) and HMO (2.5%) recorded mortality of only 27.78 and 11.11 per cent, respectively.

After 72 h of treatment, 100 per cent mortality was also recorded in 0.3 per cent methanol fraction, while neem oil emulsion (2%) and HMO (2.5%) recorded significantly lower mortality of 38.89 and 22.23 per cent, respectively.

Table 12. Effect of methanol fraction of *Acorus calamus* on adult of *Neosieulus longispinosus*

Treatment	Mortality (%)		
	24 h	48 h	72 h
Methanol fraction (0.3%)	38.89 [c] (38.51)	94.44 [a] (81.19)	100.00 [a] (88.83)
Methanol fraction (0.5%)	77.78 [b] (62.18)	100.00 [a] (88.83)	100.00 [a] (88.83)
Methanol fraction (0.7%)	100.00 [a] (88.83)	100.00 [a] (88.83)	100.00 [a] (88.83)
Neem emulsion (2%)	11.11 [d] (16.46)	27.78 [b] (31.54)	38.89 [b] (38.51)
HMO (2.5%)	5.56 [d] (8.81)	11.11 [c] (16.46)	22.23 [c] (27.82)
Control	0.00 [d] (1.17)	0.00 [c] (1.17)	0.00 [d] (1.17)
CD (0.05%)	**13.98**	**12.11**	**9.88**

Each value is mean of three replications
Figures in parentheses are arc sine transformed values
Means followed by common letter(s) do not significantly differ at P=0.05

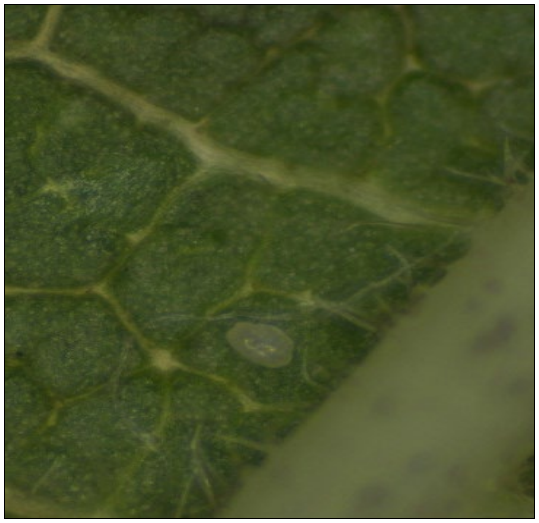

Plate 21a. Live egg **Plate 21b. Dead egg**

Plate 21. Effect of 0.7 % methanol fraction of *Acorus calamus* on eggs of *Neoseiulus longispinosus*

Plate 22a. Live adult **Plate 22b. Dead adult**

Plate 22. Effect of 0.7 % methanol fraction of *Acorus calamus* on adult of *Neoseiulus longispinosus*

4.4. EVALUATION OF SAFETY OF BOTANICAL EXTRACT TO THE ACAROPATHOGEN, *ACREMONIUM ZEYLANICUM*

Safety of methanol fraction of *A. calamus* was evaluated at three different concentrations (0.3, 0.5 and 0.7 %) in the laboratory against acaropathogen, *Acremonium zeylanicum*, along with 2 per cent neem oil emulsion and 2.5 per cent HMO and the result is presented in Table 13.

The treatment, HMO (2.5%) showed significantly higher inhibition of the acaropathogen recording 76.29 per cent. Among the different concentrations of methanolic fraction of *A. calamus* evaluated, 0.7 per cent fraction recorded significantly higher inhibition recording 62.96 per cent, followed by 0.5 per cent fraction and 0.3 per cent fraction recording 54.07 and 40.37 per cent respectively. Least per cent inhibition of 33.33 per cent was recorded by the treatment, neem oil emulsion (2%) (Plate 23).

Table 13. Effect of methanol fraction of *Acorus calamus* on acaropathogen, *Acremonium zeylanicum*

Treatment	Radial growth (cm)	Inhibition over control (%)
Methanol fraction (0.3%)	5.37	40.37 [d]
Methanol fraction (0.5%)	4.13	54.07 [c]
Methanol fraction (0.7%)	3.33	62.96 [b]
Neem oil emulsion (2%)	6	33.33 [e]
HMO (2.5%)	2.13	76.29 [a]
Control	9	0

Each value is mean of three replications

Plate 23a. Methanol fraction 0.3%

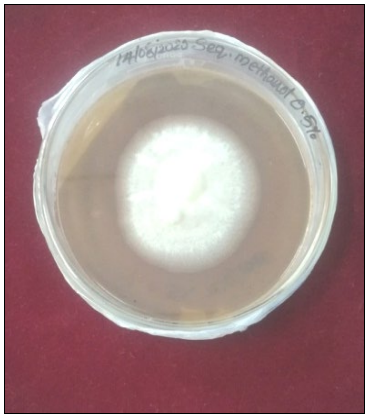

Plate 23b. Methanol fraction 0.5%

Plate 23c. Methanol fraction 0.7%

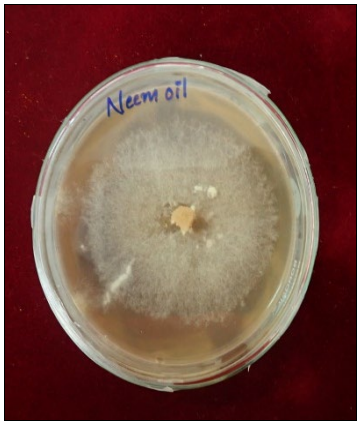

Plate 23d. Neem oil 2%

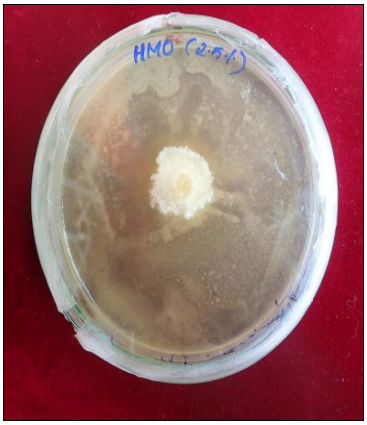

Plate 23e. Horticultural mineral oil 2.5%

Plate 23f. Control

Plate 23. Growth inhibition of *Acremonium zeylanicum* in SDAY media poisoned with methanol fraction of *Acorus calamus*

Discussion

5. DISCUSSION

The main findings of the study on efficacy of botanicals against the red spider mite, *Tetranychus truncatus* are disscussed in this chapter in the light of available literature.

5.1. LABORATORY BIOASSAY OF BOTANICALS AGAINST *TETRANYCHUS TRUNCATUS*

In the present study, crude aqueous as well as methanol extracts of ten botanicals were evaluated at various concentrations against egg and adult of *T. truncatus.*

5.1.1. Laboratory evaluation of aqueous extract of botanicals

Among the ten aqueous botanical extracts evaluated in the present study, none of the extracts showed ovicidal action against *T. truncatus* eggs at the three concentrations evaluated *viz.*, 5, 7.5 and 10 per cent. Hatchability of 100 per cent was observed in all the botanical treatments. However, the botanical extracts differed significantly in their adulticidal action against the mite. Similar observation was made in the laboratory study conducted by Radhakrishnan and Prabhakaran (2014), where aqueous extracts of different botanicals viz., *Ocimum basilicum, Lantana camara, Ageratum houstonianum, Allamanda cathartica, Bidens pilosa, Casuarina equisetifolia, Conyza bonariensis, Crassocephalum crepidioides, Glyricidia sepium and Tithonia diversifoila* showed 100 per cent adult emergence of red spider mite, *Oligonychus coffeae,* when tested at concentrations of 2.5 and 5 per cent. They also reported that the botanicals varied in their action against adult mite. The reason for lack of ovicidal action might be because the botanical extracts lack the ability to penetrate the chorion of eggs, but adults were affected due to the contact action (Radhakrishnan and Prabhakaran, 2014). According to Sarmah *et al*. (2009), aqueous extract of *Acorus calamus* caused 70.62 per cent mortality of *Oligonychus coffeae* eggs at 10 per cent concentration, which is contradicts the findings of the present

study.

The present study showed that aqueous extracts of *Annona squamosa*, *C. papaya* and *B. monnieri* had significant acaricidal activity against the adult mite. After five days of treatment, the seed extract of *A. squamosa* and leaf extract of *C. papaya* at 10 per cent concentration recorded the highest mortality of adult mites (98.67%). The lower concentrations of *A. squamosa* (7.5 and 5 %) as well as 5 per cent leaf extract of *C. papaya* and whole plant extract of *B. monnieri* also caused significant mortality of adults (Fig. 1.).

Field evaluation of aqueous seed extract of *Annona squamosa* (10%) was reported to cause significant reduction in population of two species of flea beetles viz., *Podagrica uniforma* and *P. sjostedti* infesting okra (Onunkun, 2012).

The seed oil of *A. squamosa* was reported to be very effective in killing the Kanzawa spider mite, with more than 80 per cent mortality three days after application of 0.5 or 0.25 per cent seed oil. The mortality rates increased to more than 90 per cent in five days, and by the 10^{th} day all the spider mites tested were killed. Even at 0.125per cent, the oil was able to cause 100 per cent mortality of spider mites in 10 days (Lin *et al*., 2009). However, the aqueous leaf extract of *A. squamosa* (10%) was reported to cause only 40 per cent mortality of *T. urticae* adults after 72 h of treatment under laboratory condition (Premalatha *et al*., 2018).

The acaricidal effect of *Annona* sp. is attributed to structurally diversifed secondary metabolites. The extracts of bark, branches, leaves, fruits, and seeds contain alkaloids, acetogenins, diterpenes and flavonoids (Gajalakshmi *et al*., 2011). The active substances of *Annona* sp. was reported to affect the neuroendocrine system and thereby interfere the process of metamorphosis (Freitas *et al*., 2014).

Sunarti (2019), reported that *C. papaya* leaf extract caused 100 and 90 per cent mortality of tomato aphid at concentrations of 60 and 45 per cent, respectively, after 48 h of treatment. This may be due to the presence of active substances like papain and flavanoid present in papaya leaves, which act as stomach poison and nerve

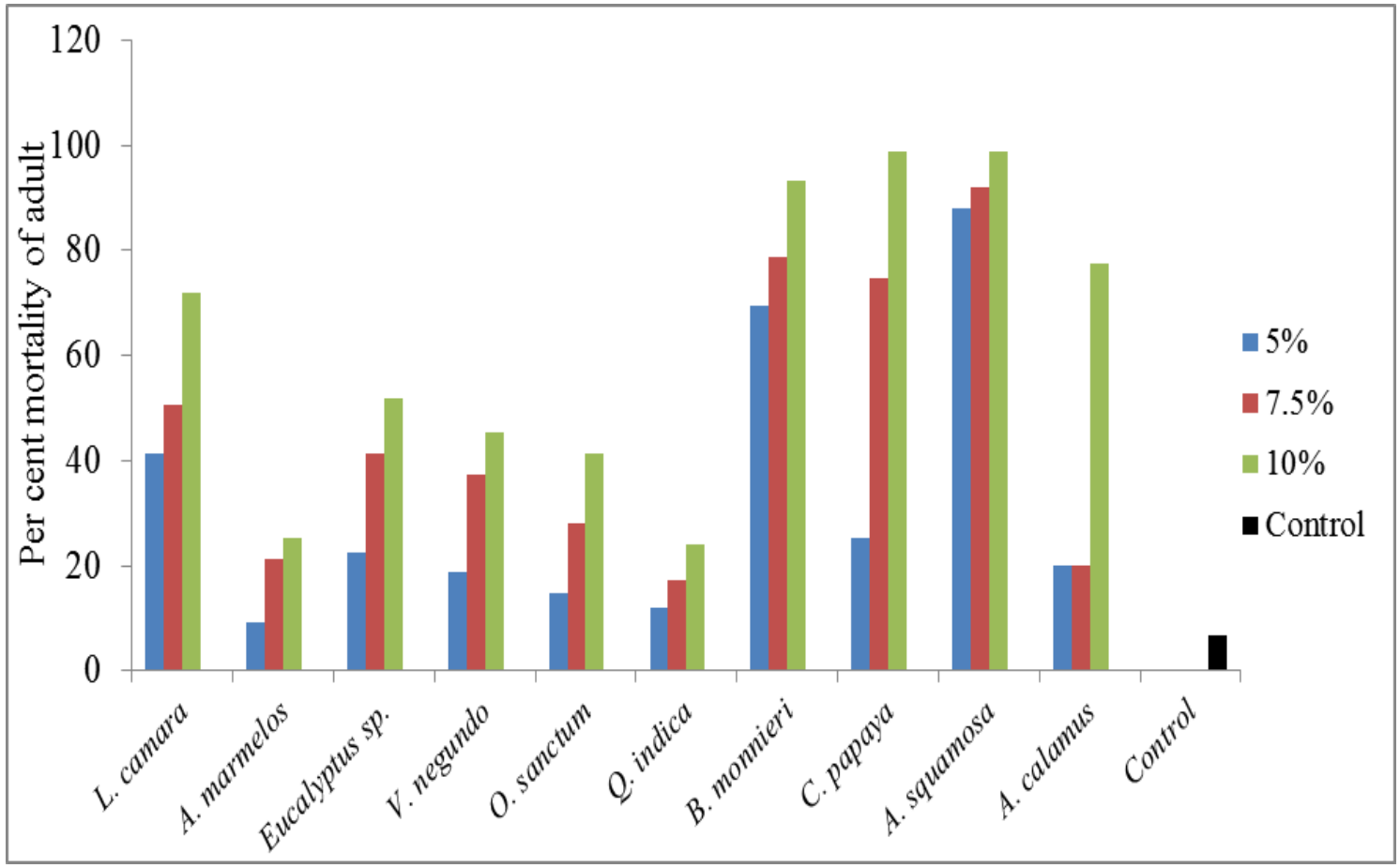

Fig. 1. Effect of aqueous extract of different botanicals on adults of *Tetranychus truncatus*

poison respectively. Also, the saponins present in papaya leaves were reported to cause disruption in moulting process in insects (Setiawan and Oka, 2015). Though the acaricidal property of *C. papaya* had not been reported earlier, the present study identifies the potential of aqueous extract of *C. papaya* in bringing about significant mortality on adults of *T. truncatus.*

In this study, the whole plant aqueous extract of *Bacopa monnieri* showed significant adulticidal action against *T. truncatus*. However, no literature pertaining to acaricidal/insecticidal properties of the plant extracts could be found. But, Liu *et al.* (2020) studied the composition of essential oil of another species of *Bacopa namely, B. caroliniana* and its insecticidal activity against the rice weevil, *Sitophilus oryzae*. Among 18 volatiles recorded, α-terpinolene was the major compound and the volatiles caused inhibition of acetylcholine esterase activity. This could result in the accumulation of acetylcholine at synapse and permanent stimulation of post synaptic membrane, thus affecting the neuromuscular co-ordination and cause death of the insects.

In the present study, whole plant extract of *Lantana camara* (10%) showed significant mortality of 72.00 per cent of the adult mite. This result can be supported by the study of Al-Mamun *et al*. (2015) who evaluated *L. camara* at 10 per cent concentration and reported mortality of 79.31 per cent against adult red spider mite, *Oligonychus coffeae* after 72 h of treatment. The active ingredients responsible for acaricidal property of *Lantana camara* are α-Cubebene and δ-Cadinene.

Leaf extracts of *Eucalyptus* (10%), *Vitex negundo* (10%), and *Ocimum sanctum* (10%) also showed appreciable adulticidal activity against the mite. The botanicals recorded comparable mortality of 52.00, 45.00 per cent and 41.33 per cent respectively at 120 h. Similar results were obtained in the study conducted by Sathyaseelan *et al*. (2020) who evaluated the efficacy of leaf extracts of certain native botanicals against *T. urticae* on horse gram in the laboratory and found that the extracts of *V. negundo* and *Eucalyptus* sp. resulted in 61.88 and 55.00 per cent mortality, respectively, within 48 h of treatment. Ong *et al*. (2018) also reported

acaricidal effect of crude aqueous fraction of *V. negundo* (37.77%) against larvae of the tick *Rhipicephalus sanguineus*, recording 57.43 per cent mortality after 24 h of treatment, with LC_{50} value of 31.91 per cent. The leaf extract of *Ocimum tenuiflorum* at 3 per cent concentration was reported to cause 82.2 per cent mortality of *T. neocaledonicus* (Roy *et al.,* 2011).

5.1.2. Laboratory evaluation of methanol extract of botanicals

Among the different botanicals evaluated, mortality of eggs was recorded only by 2 per cent methanol extract of *A. squamosa* as well as *Acorus calamus* and *Aegle marmelos*. However, the highest mortality recorded was only 12 per cent. But the methanol extract resulted in significant mortality of adult mite. The reason for low egg mortality of methanol extract of the botanicals may also be due to the inability to penetrate the chorion of egg as mentioned in 5.1.1.

The result could be corroborated with the study conducted by Erdogan *et al.* (2012), who evaluated ethanol extract of five plant extracts *viz.*, *Allium sativum*, *Rhododendron luteum*, *Helichrysum arenarium*, *Veratrum album* and *Tanacetum parthenium* at different concentrations against the eggs of *Tetranychus urticae*, where all the treated eggs hatched. However, these extracts showed significant mortality in adult mites.

In the present study, direct methanol extract of botanicals were found to be more effective against the adults of *T. truncatus* when compared to eggs. Methanol extract of *A. calamus* and *C. papaya,* at the two concentrations (1 and 2 %) evaluated resulted in 100 per cent mortality of the adults (Fig. 2.). In dose response relationship study conducted by Kumar *et al.* (2015), evaluation of ethanolic rhizome extract of *A. calamus* recorded 91.11 and 100 per cent mortality against adult male as well as female of *Drosophila melanogaster* respectively, at concentration of 100 mg/ ml after 24 h of treatment. When tested against larvae at the same concentration, it caused only 38.89 per cent mortality.

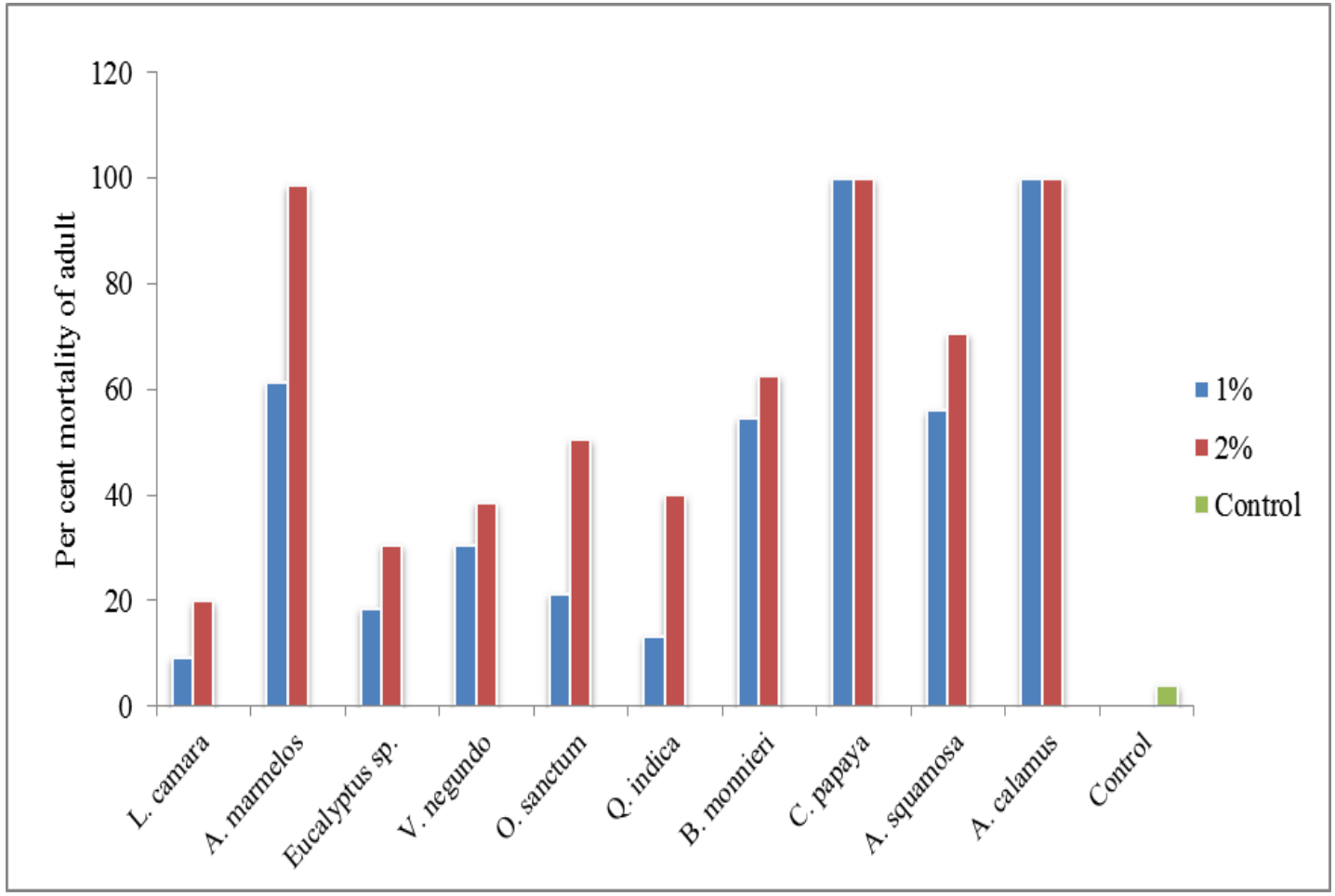

Fig. 2. Effect of methanol extract of botanicals on gravid females of *Tetranychus truncatus*

Shukla *et al.* (2009) studied the efficacy of leaves and rhizomes of *A. calamus* using two different solvents (methanol and petroleum ether) against the pulse beetle, *Callosobruchus chinensis*. Coating 1g of seeds with 0.4g of methanolic leaf extract recorded 100 per cent and that of rhizome extract recorded 92.4 per cent mortality of adult insects, after 24 h of treatment. However, at the same concentration, petroleum ether extracts of rhizome and leaves recorded 92.0 and 69.9 per cent mortality of adults respectively.

The biologically active compounds present in rhizomes of *Acorus* spp. causing insecticidal activities were identified as cis-asarones, trans-asarones as well as phenylpropenes through spectroscopic analysis. Also, asarone is abundant in essential oil of *A. calamus,* which acts as antifeedant and growth inhibitors. The insecticidal activity of *Acorus* sp. may be because of individual or combined effect of these bioactive compounds (Balakumbahan *et al*., 2010). The same compounds might also have acaricidal effect, which resulted in significant mortality of *T. truncatus* in the present study.

Rahayu *et al*. (2020) studied the potency of ethanolic leaf extract of papaya against standard as well as field population of German cockroach, *Blattella germanica*. The residue that could cause 90 per cent mortality (LR_{90}) of adults ranged from 6.05 - 8.92 mg/cm^2 and the time required to record same mortality with 9.00 mg/cm^2 residue ranged from 3.58 - 5.83 h. Also, a very high level of repellency ranging from 88.89 - 94.74 per cent after 24 h was recorded. In the present study, the methanol extract of papaya leaf showed significant acaricidal property, which has not been reported earlier.

Methanol extract of *Aegle marmelos* at 2 per cent concentration also recorded significant mortality of the adult mite recording 98.67 per cent. The result is in agreement with the reports of Murasing *et al*. (2017), where four solvent extracts *viz*., petroleum ether, methanol, ethanol and water extract of *A. marmelos* at 5 per cent concentration were evaluated against *Callosobruchus chinensis,* recording highest mortality in petroleum ether recording 82 per cent, followed by methanol, ethanol and

water extracts recording 80, 76 and 74 per cent mortality, respectively.

During the present study, after 120 h of treatment, methanol extract of *A. squamosa* seeds at 2 per cent concentration showed significant mortality of 70.67 per cent, followed by *B. monnieri*. In a study conducted by Maciel *et al*. (2015), the solvent extracts of *Annona muricata* seeds in ethanol and hexane resulted in significant mortality of *T. urticae* recording LC_{50} values as low as 1.77 and 3.29 mg/ml respectively. Ethanolic seed extract of *A. mucosa, A. sylvatica* and *A. muricata* was reported to have significant mortality of 83, 80 and 67 per cent against adult females of *T. urticae* (Miotto *et al*., 2020). Freitas *et al*. (2014) evaluated the effect of methanolic leaf extract of *Annona dioica, A. cacans,* and *A. coriacea* on growth and reproduction of *Spodoptera frugiperda.* Significant reduction in pupal biomass, larval viability and pupal viability of *S. frugiperda*, recording 93.4 mg, 24 per cent and 64 per cent respectively, was observed when treated with *A. coriacea*. Among the three extracts, *Annona dioica* and *A. coriacea* showed high flavanoid content recording 650 and 480.45 mg gallic acid/g of extract, respectively. *A. coriacea* and *A. cacans* recorded high phenol content accounting 756.68 and 560.22 mg gallic acid/g of extract, respectively. The compound responsible for insecticidal activity of *A. squamosa* seed extract is adjacent bis-tetrahydrofuran ring acetogenins and that of *A. muricata* is mono- tetrahydrofuran ring acetogenins. Acetogenins with two tetrahydrofuran ring are found to be more effective than with a single ring (Leatemia and Isman, 2004). The seed oil of *A. squamosa* was reported to cause significant mortality of *Tetranychus kanzawai* even at a lower concentration (Lin *et al*., 2009).

In the present study, the whole plant methanol extract of *B. monnieri* also showed significant adulticidal action against *T. truncatus*. However, no literature pertaining to acaricidal/insecticidal properties of the plant species could be found. The methanol extract of *O. sanctum* leaves showed significant acaricidal property against adult *T. truncatus* when compared to aqueous extracts. The methanolic extract at 2 per cent concentration recorded 50.67 per cent mortality, whereas in aqueous extract, even at higher concentration of 10 per cent, mortality of only 41.33 per cent was observed. This result agrees with the findings of Roy *et al.* (2011), who evaluated

aqueous as well as methanol extracts of three botanicals against *T. neocaledonicus* and found that, the methanol extract of *Ocimum tenuiflorum* resulted in a higher mortality of 93.3 per cent, compared to 82.2 per cent mortality in aqueous extract at 3 per cent concentration.

5.2. IDENTIFICATION OF BIOACTIVE SOLVENT FRACTION OF *ACORUS CALAMUS*

5.2.1. Laboratory evaluation of solvent fractions of *Acorus calamus* by liquid liquid partitioning

Acorus calamus was fractionated using three solvents *viz.* hexane, chloroform and methanol based on the increasing order of their polarity and efficacy of the fractions were evaluated at three different concentration (0.3, 0.5and 0.7 %) against *T. truncatus*. Among the different fractions, ovicidal action was recorded only by methanol fraction (Fig. 3.). Cent per cent mortality was recorded by 0.7 per cent methanol fraction, while lower concentration of 0.5 per cent recorded only 29.33 per cent mortality. Sarmah *et al.* (2007) evaluated the ovicidal action of three different solvent fractions of *A. calamus* namely, petroleum ether, acetone and methanol fraction against *Oligonychus coffeae.* Significantly higher ovicidal action was recorded by petroleum ether fraction followed by acetone fraction compared to methanol fraction at concentrations of 0.5, 1.0 and 2.0 per cent. However, at higher concentration of 5 per cent, methanol fraction also recorded significant reduction in hatchability of 97.7 per cent.

In the present study, 100 per cent mortality of adult mites was observed in methanol fraction of *A. calamus* (0.5 and 0.7 %), 24 h after treatment. By fourth day of treatment, all treatments showed 100 per cent mortality of adult mites (Fig. 4.). The present study is in line with the study conducted by Singh (2015), who evaluated aqueous, methanol, hexane and ethyl acetate extracts of *A. calamus*, *V. negundo, Adathoda vasica* and *Dioscorea deltoidea* against second instar larvae of *Helicoverpa armigera* as well as *Plutella xylostella*. Among the solvent extracts, methanol extract

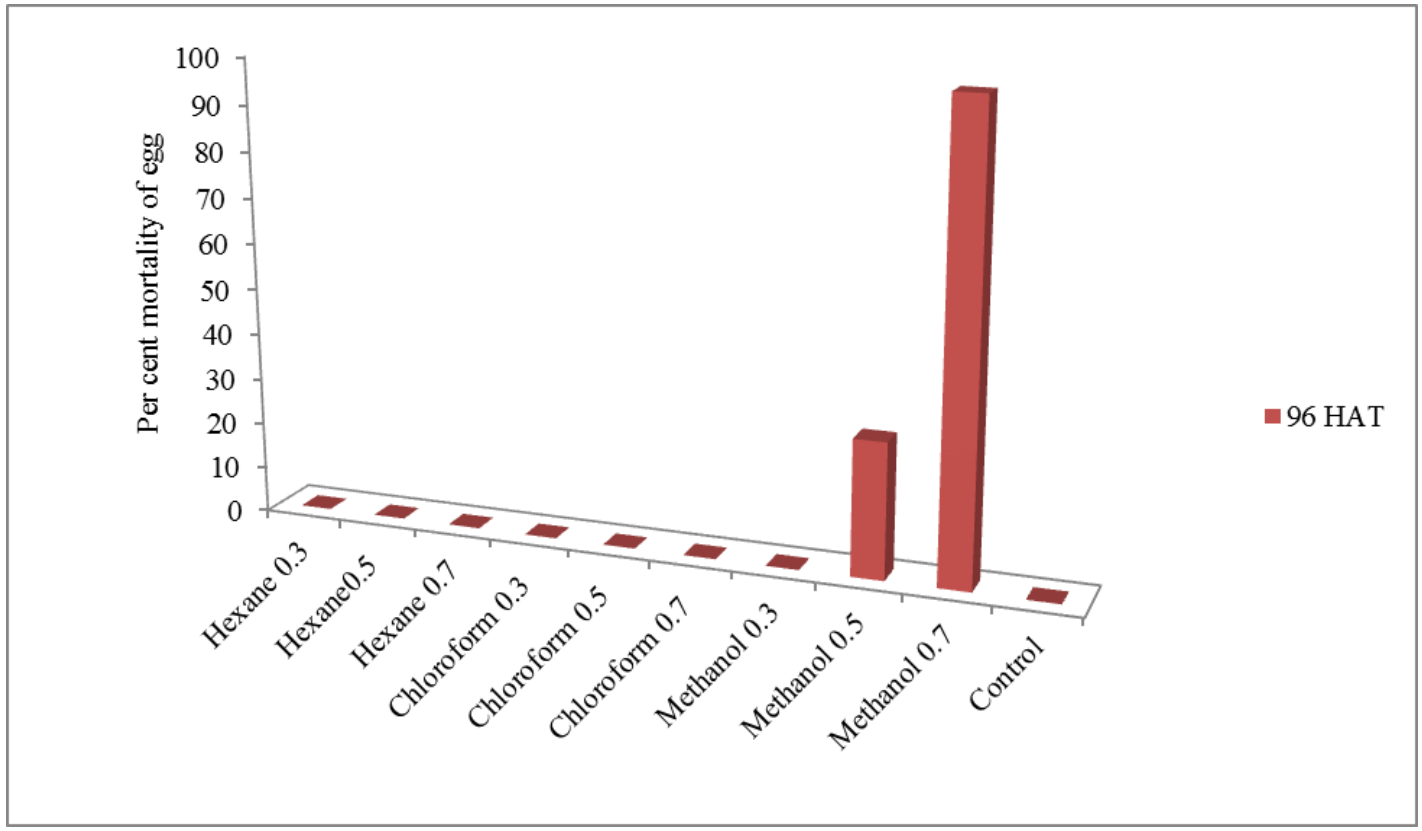

Fig. 3. Effect of *Acorus calamus* solvent fractions on eggs of *Tetranychus truncatus*

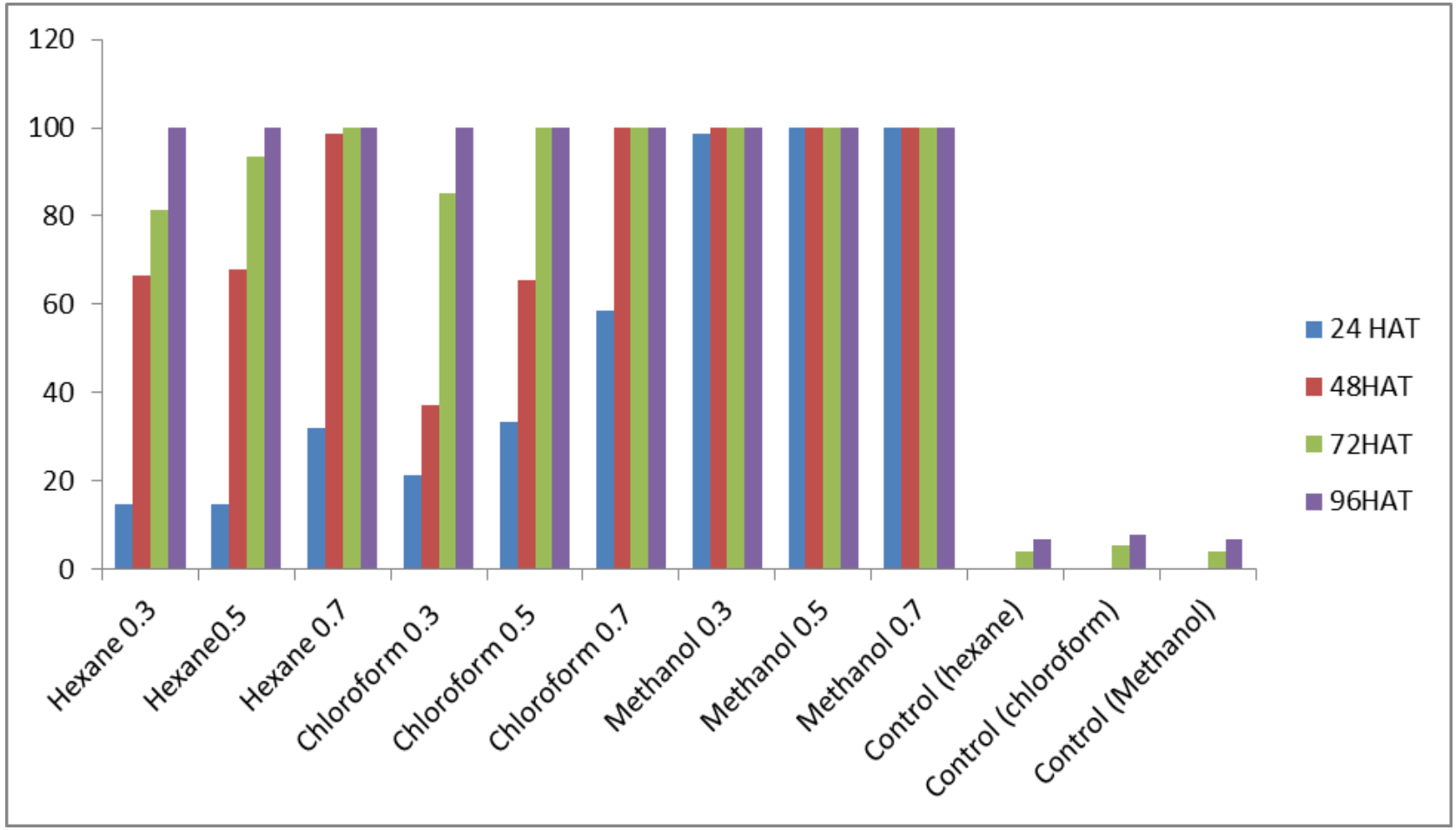

Fig. 4. Effect of *Acorus calamus* solvent fractions on adults of *Tetranychus truncatus*

of all the four plants showed highest toxicity against the insects, and among the botanicals, *A. calamus* extracts caused highest mortality of both *H. armigera* and *P. xylostella*. Lowest LC_{50} value of 1.75 and 2.85 per cent was recorded by the methanol extract of *A. calamus* for *H. armigera* and *P. xylostella*, respectively. Egg hatchability was also lowest in methanol extract of *A. calamus* recording 32.46 and 54.28 per cent, against *P. xylostella* and *H. armigera*, respectively.

5.2.2. Evaluation of bioactive solvent fraction of *Acorus calamus* against *Tetranychus truncatus* in polyhouse

The methanol fraction of *A. calamus*, one of the bioactive fractions identified in the study was evaluated for efficacy against *T. truncatus* on cucumber in polyhouse along with the aqueous extract of *Annona squamosa* (7.5%), neem oil (2%), horticultural mineral oil (HMO 2.5%) and a novel acaricide, spiromesifen. *Acorus calamus* recorded significant reduction in mite population compared to HMO and neem oil within one day of treatment application. Cent per cent reduction in mite population was recorded by spiromesifen on seventh day, while *Acorus calamus* 0.5 per cent recorded complete reduction in mite population by tenth day. By fourteenth day *Acorus calamus* at 0.3 concentration and HMO also recorded cent per cent reduction in mite population. However, by fourteenth day the efficacy of all the treatments was comparable with each other (Fig. 5.).

Efficacy of neem oil and HMO against spider mites has been reported by many workers (Bernardi *et al.*, 2013; Kavya *et al*., 2015; Roy *et al*., 2015; Premalatha and Chinniah, 2017; Raghavendra *et al*., 2017; Nag *et al*., 2020). In the present study, the efficacy of methanol fraction of *A. calamus* was found to be superior to HMO and neem oil and on par with the novel acaricide, spiromesifen. The efficacy of crude aqueous extract of *Annona squamosa* was also comparable with HMO, neem oil and spiromesifen. The results of the polyhouse study suggest that the botanical *A. calamus* and *A. squamosa* can be potential candidates for exploitation in the management of spider mites under protected cultivation.

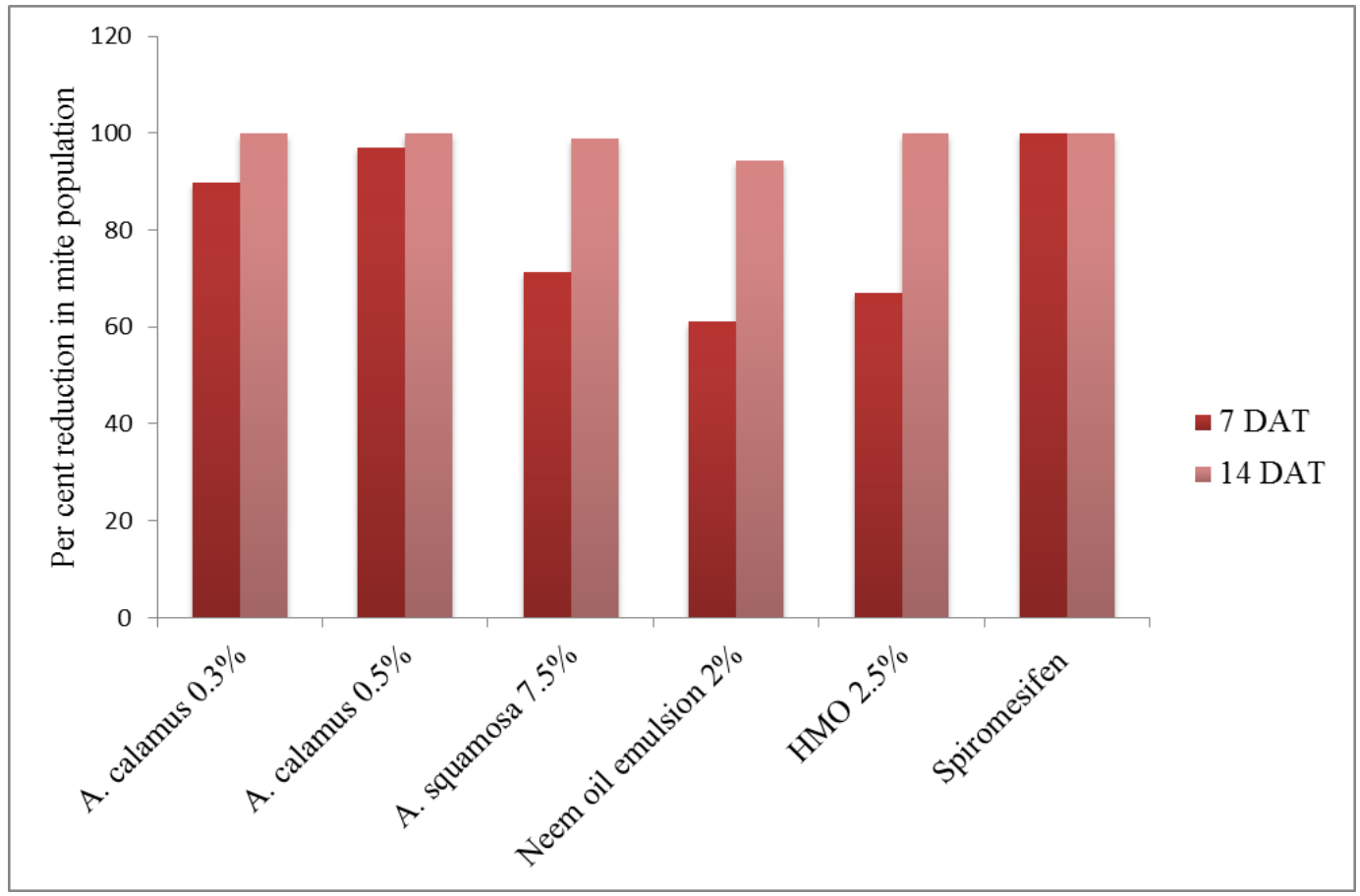

Fig. 5. Per cent reduction of *Tetranychus truncatus* population on cucumber in polyhouse

In laboratory bioassay studies, methanol fraction of *A. calamus* at 5 per cent concentration was reported to cause 94.6 per cent mortality of adult red spider mite *Oligonychus coffeae*. The ethanol fraction of *A. calamus* was also proved to be effective against spider mites (Sarmah *et al*., 2007). Premalatha and Chinniah (2017) evaluated 10 per cent ethanolic extracts of *Vitex negundo*, *Ocimum sanctum*, *Citrullus colocynthis*, *A. calamus*, along with rosemary oil, neem oil, citronella oil, lemon grass oil and fenpyroximate against *T. urticae* on tomato under field condition. Among the treatments, *O. sanctum*, *V. negundo* and *A. calamus* showed significantly higher per cent reduction of eggs recording 79.90, 79.72 and 79.54 per cent and these extracts exhibited same trend in per cent reduction of adults and nymph population, recording 78.50, 78.38 and 78.23 per cent respectively.

The methanol fraction of *A. calamus* was also reported to have significant insecticidal action against many insect pests in field and storage. The methanol fraction of *A. calamus* was found promising in reducing the field population of *Plutella xylostella* on cabbage and was superior to the methanol extracts of the botanicals, *V. negundo* and *Adhatoda vasica* (Singh, 2015). After 15 days of spraying, the methanol extract of *A. calamus* recorded the highest reduction in mean population of *P. xylostella* (54.25 %) followed by methanol extract of *V. negundo* and hexane extract of *A. calamus* both recording 33.82 per cent reduction in field population.

Acorus calamus fractionated with petroleum ether, acetone and methanol was evaluated against two stored grain insects, *Tribolium confusum* and *Sitophilus oryzae*. Methanol fraction did not show any effect on *Tribolium confusum* at different concentration evaluated. However, methanol fraction at 0.471 mg/cm^2 was found to cause 99 per cent mortality of *Sitophilus oryzae*, after 48 h of treatment (Hossain *et al.*, 2008).

Lee *et al*. (2002) evaluated different fractions of *Acorus gramineus* using hexane, chloroform and ethyl acetate as solvents at 2500 ppm against female adults of *Nilaparvata lugens* and *Myzus persicae* as well as third instar larvae of *Spodoptera litura* and *Plutella xylostella*. Among the fractions, hexane fraction recorded highest

significant mortality against all insects evaluated and recorded 100 per cent mortality against *N. lugens* and *P. xylostella*. The efficacy of *A. gramineus* fractions depends on the concentration of bioactive compound *i.e*. *cis*-asarone and *trans*-asarone present. At 2000 ppm both *cis* and *trans* asarone cause 100 per cent mortality of *N. lugens* and *P. xylostella* but, mortality reduces with decrease in concentration.

In the present study, the aqueous seed extract of *Annona squamosa* was found effective in bringing down the population of *T. truncatus* on cucumber in polyhouse. Though studies on the efficacy of seed extracts of *A. squamosa* against mite pests are not conducted earlier, some studies document its efficacy against insect pests. Onunkun (2012) conducted a study to evaluate the aqueous extracts of *Jatropha curcas, Vernonia amygdalina, Ageratum conyzoides, Chromolaena odorata* and *Annona squamosa each* at 10 per cent concentration, against the flea beetles, *Podagrica uniforma* and *P. sjostedti* on okra under field condition. *A. squamosa* extract recorded significant reduction in the population of the flea beetles consistently during the two seasons, 2008 and 2009.

5.3. SAFETY OF BIOACTIVE SOLVENT FRACTION OF *A. CALAMUS* AGAINST *NEOSEIULUS LONGISPINOSUS*

In the present study, the eggs of the predator, *N. longispinosus* treated with methanol fractions of *A. calamus* at 0.7 per cent concentration did not hatch even after fourth day of treatment (Fig. 6.). The methanol fraction at 0.7 per cent recorded 100 per cent mortality of adult *N. longispinosus* within one day of treatment. By third day all the three concentrations of methanol fraction evaluated recorded complete mortality of adult *N. longispinosus* (Fig. 7.). The results indicate that the methanolic fraction of *A. calamus* is not safe to the mite predator, *N. longispinosus*.

Literature on the safety of *A. calamus* extracts to predatory mites is scanty. However few studies have reported safety of some botanical extracts to natural enemies of mites as well as insect pests. Aqueous extracts of *Xanthium strumarium, Acorus calamus* and *Polygonum hydropiper* recorded significant reduction in the

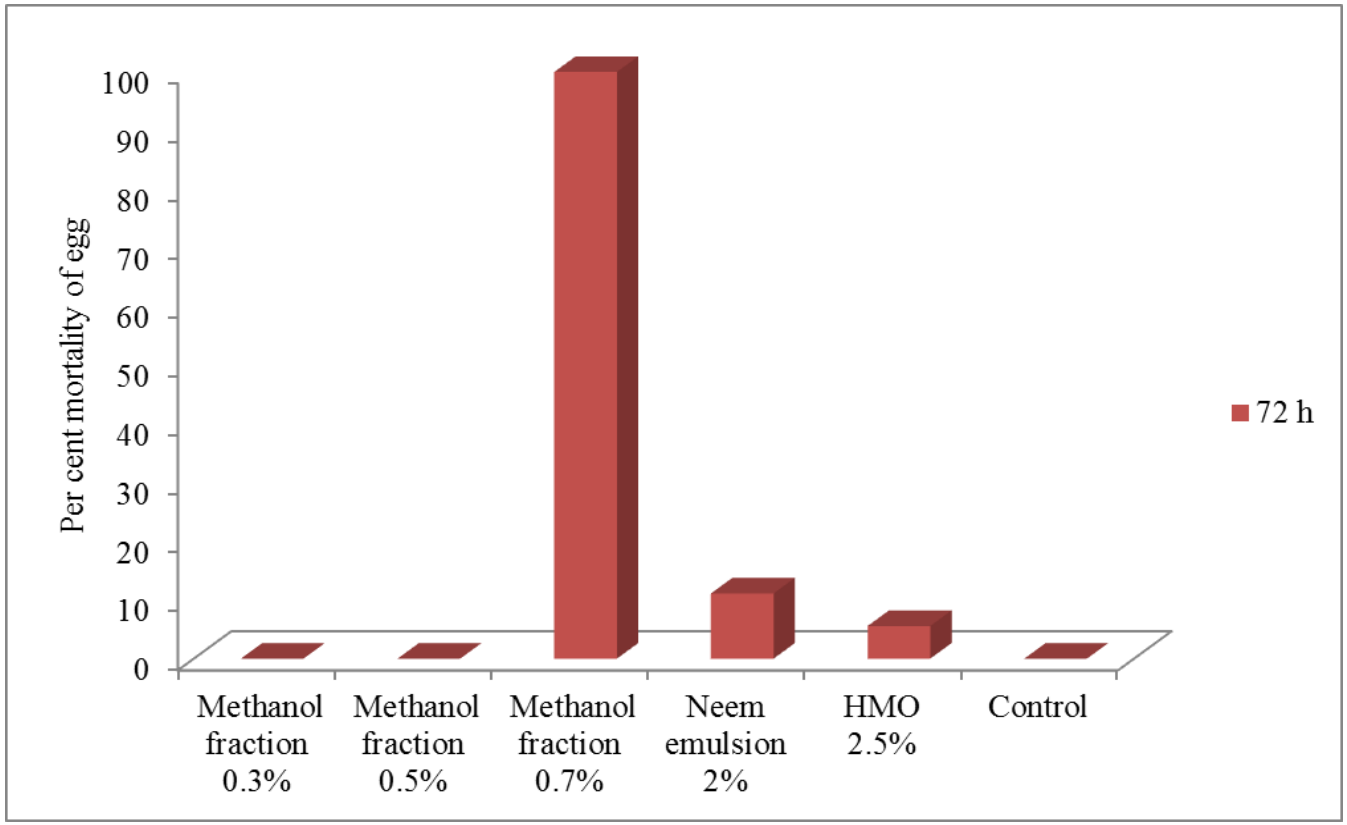

Fig. 6. Effect of methanol fraction of *Acorus calamus* on egg of *Neosieulus longispinosus*

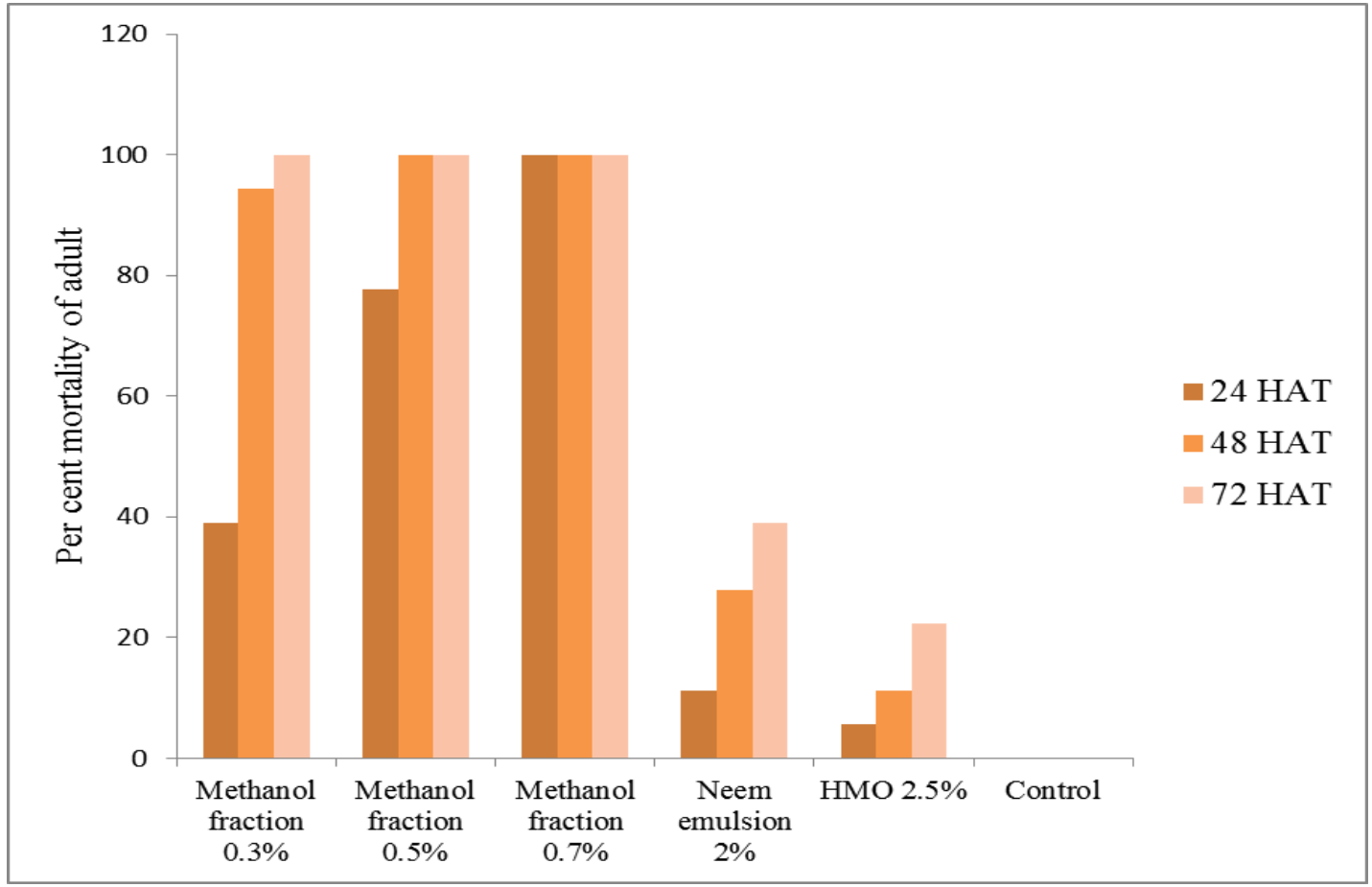

Fig. 7. Effect of methanol fraction of *Acorus calamus* on adults of *Neosieulus longispinosus*

feeding potential of the predatory coccinellid, *Stethorus gilvifrons* at 10% concentration, however, no mortality was observed until 14 days in any of the treatments (Sarmah *et al*, 2007).

Salman *et al.* (2018) reported the toxicity of *Salvia officinalis* (sage) and *Rosmarinus officinalis* (rosemary) extracts against two important predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis,* which resulted in significant mortality of the predators after 72 h of exposure. The aqueous leaf extracts of *Clerodendron inermae* and *V. negundo* at five per cent concentration were reported to reduce the field population of the predators, *Coccinella septempunctata* and *Amblyseiu*s sp., significantly, compared to neem based botanical pesticides (Smitha and Giraddi, 2006).

The methanolic extracts of the botanicals, *Piper retrofractum* and *Annona squamosa* (RS) at 0.1 per cent concentration did not affect the level of parasitization by two hymenopteran parasitoids namely, *Diadegma semiclausum* and *Eriborus argentiopilosusas* on the caterpillars of *Crocidolomia pavonana* and *Plutella xylostella* in cabbage field. At the same concentration, methanolic extracts of *Aglaia odorata* and *A. squamosa* (OS), considerably reduced parasitization by *E. argentiopilosus* (Dadang *et al*., 2009).

5.4. SAFETY OF BIOACTIVE SOLVENT FRACTION OF *A. CALAMUS* AGAINST THE ACAROPATHOGEN, *ACREMONIUM ZEYLANICUM*

During the present study, methanolic fraction of *A. calamus* even at the lowest concentration evaluated, recorded an inhibition of 40.37 per cent and thus revealed the antagonistic effect to the acaropathogen *A. zeylanicum* (Fig. 8.). The result of the experiment suggests that methanolic fraction of *A. calamus* at 0.3, 0.5 and 0.7 per cent is not compatible with *A. zeylanicum*.

Literature pertaining to safety of *A. zeylanicum* to botanical fraction is unavailable. However, few literatures on safety of some botanical extracts and essential oils are cited below to support this study.

Essential oil from fresh infusions of oregano, laurel and rosemary leaves were found incompatible to *Acremonium* sp. Oregano essential oil showed complete inhibition in growth of the fungus at 20 per cent concentration. At the same concentration, rosemary and laurel leaves also inhibited the fungal growth by 63 and 44 per cent , respectively (Racowski *et al*., 2016).

Growth of another species of *Acremonium viz*., *Acremonium apii* was inhibited by ethanolic extract as well as essential oil of *Ageratum conyzoides, Callistemon citrinus, Cymbopogon citratus* and *Ocimum gratissimum.* The essential oil of *O. gratissimum* at 400 ppm and ethanolic extract of *C. citrinus* at 10,000 ppm exhibited highest significant inhibition of radial growth recording 100 and 77.68 per cent against *A. apii* (Sandrine *et al*., 2013).

Sahayaraj *et al.* (2011) reported that the methanolic seed extract of *A. squomosa* caused significant inhibition (30.94 %) of the entomopathogenic fungus, *Beauveria bassiana* compared to the aqueous extract (19.69 %) at different concentrations. However, solvent leaf extracts of botanicals, *Syndrella nodiflora*, *Premna tomentosa*, *Vitex negundo*, *Ipomea carnea*, *Pteridium aquilinum* were compatible with the fungus.

Horticulture mineral oil (HMO) at 2.5 per cent concentration was reported to inhibit the acaropathogen *A. zeylanicum* by 46.67 per cent *in vitro* (Yadav, 2018). However, in this study, at the same concentration HMO caused significantly higher inhibition of 76.29 per cent.

It was reported that the secondary metabolites present in botanical extracts cause inhibition of growth in fungus by affecting the mycelial growth, spore germination and enzyme production. The secondary metabolites also inhibit the germ

tube elongation and cause delayed sporulation (Lengai *et al*., 2020).

The study identified appreciable acaricidal property of the botanicals: *Acorus calamus*, *Carica papay*a, *Annona squamosa*, *Bacopa monnieri*, *Aegle marmelos* and *Lantana camara* against spider mites. The methanol fraction of *A. calamu*s and crude aqueous extract of *A. squamosa* emerged as a promising candidate in mite pest management under protected cultivation. However, further studies on qualitative phytochemical analysis and field evaluation have to be conducted to identify the bioactive component in these botanicals.

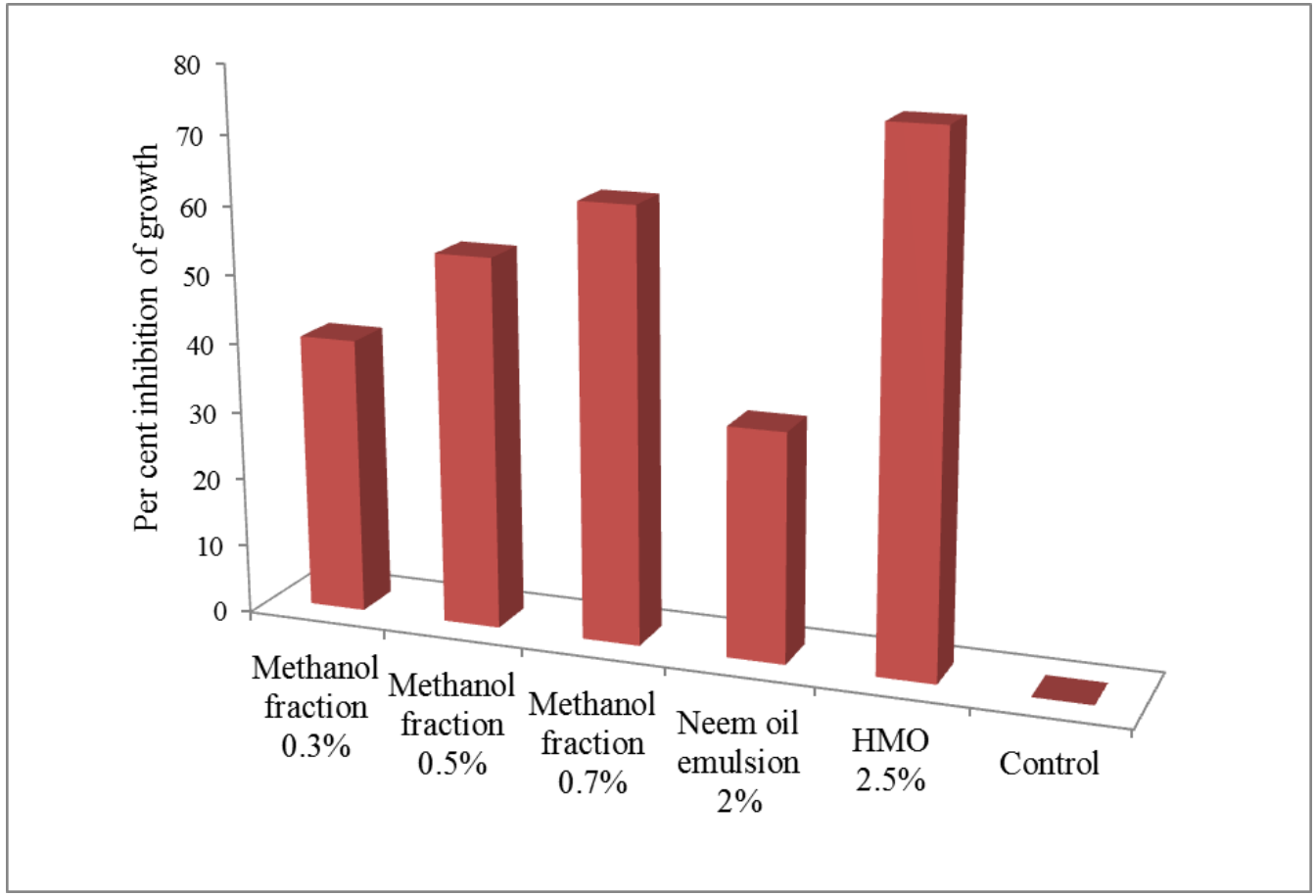

Fig. 8. Effect of methanol fraction of *Acorus calamus* on acaropathogen, *Acremonium zeylanicum*

Summary

6. SUMMARY

The study entitled "Bioefficacy of botanicals against the spider mite, *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)" was conducted in the Acarology laboratory, Department of Agricultural Entomology, College of Horticulture, KAU, Vellanikkara during 2019-2020. Ten plants *viz*., *Acorus calamus, Bacopa monnieri, Quassia indica, Eucalyptus* sp.*, Lantana camara, Aegle marmelos, Annona squamosa, Vitex negundo, Carica papaya* and *Ocimum sanctum* were evaluated for their efficacy against *Tetranychus truncatus* Ehara. The salient findings of the study are summarized below.

- Efficacy of aqueous extracts of the ten botanicals was evaluated against egg and adult of *Tetranychus truncatus* at three different concentrations namely, 5, 7.5 and 10 per cent. Aqueous extracts of none of the plants showed ovicidal action and recorded 100 per cent hatchability by 120 h of treatment. However, the treatments differed significantly in their adulticidal action against the mite. Among the extracts evaluated seed extract of *A. squamosa* (98.6%), leaf extract of *C. papaya* (98.6%) and whole plant extract of *B. monnieri* (93.3%) exhibited significantly higher mortality of adult mite at 10% concentration. The lower concentrations of *A. squamosa* (7.5 and 5 %), also caused significant adulticidal action recording 92.00 and 88.00 per cent mortality, respectively. Appreciable mortality of adult mites were also recorded in the following treatments; *B. monnieri* (7.5%), *A. calamus* (10%), *L. camara* (10%) and *B. monnieri* (5%) recording 78.67, 77.33, 72.00 and 69.33 per cent, respectively.

- The yield of methanol extract of all the ten botanicals were recorded and maximum yield of 7.14 per cent was recorded in seed extracts of *A. squamosa,* followed by *A. calamus* (5.82%).

- Efficacy of methanol extracts of the botanicals was evaluated against eggs and adults of *T. truncatus* at two different concentrations *viz.*, 1 and 2 per cent. The

crude methanolic extracts showed only negligible ovicidal action, the highest significant mortality being 12 per cent recorded by 2 per cent extract of *A. squamosa* and *A. calamus*. However, methanol extracts of the botanicals were found effective against the adults of *T. truncatus*. Methanol extracts of *A. calamus* and *C. papaya,* showed 100 per cent mortality of the adults at both concentrations (1 and 2%) evaluated followed by *A. marmelos* (2%) which recorded 98.67 per cent mortality. Appreciable mortality of adult mites were also observed in the treatments *A. squamosa* (2%), *B. monnieri* (2%) and *A. marmelos* (1%) recording 70.67, 62.67 and 61.33 per cent, respectively.

- *Acorus calamus*, one of the promising botanicals identified from laboratory bioassay studies, was fractionated using three different solvents (hexane, chloroform and methanol) and evaluated against egg and adult of *T. truncatus* separately at 0.3, 0.5 and 0.7 per cent concentration. The yield of hexane, chloroform and methanol fractions of *A. calamus* rhizome recorded 3.91, 3.48 and 4.48 per cent, respectively. Among the different fractions, methanol fraction (0.5 and 0.7 %) only showed ovicidal action recording mortality of 100 and 29.33 per cent, respectively. Within a day of treatment application, 100 per cent mortality of adult mites was recorded in *A. calamus* methanol fraction at 0.5 and 0.7 per cent concentration. All the solvent fractions evaluated, showed 100 per cent mortality of adult mites by fouth day of treatment.

- The bioactive solvent fraction of *A. calamus viz*., methanol fraction (0.3 and 0.5%) was evaluated for efficacy against *T. truncatus* on cucumber in polyhouse, along with aqueous extract of *A. squamosa* (7.5%), neem oil emulsion (2%), horticultural mineral oil (2.5%) and spiromesifen (100 g a.i/ ha). Seven days post treatment, the methanol fraction (0.3 and 0.5 %) showed mean population reduction of 89.68 and 97.04 per cent, respectively and was found comparable with spiromesifen. After 10 days of treatment, 0.5 per cent methanol fraction recorded complete reduction in mite population. By 14th day 0.3 per cent methanol fraction also recorded complete reduction in mite

population. All the treatments evaluated were found comparable with spiromesifen after 14 days of treatment.

- Methanol fraction of *A. calamus* at 0.3, 0.5 and 0.7 per cent concentration when evaluated for safety against eggs and adults of predatory mite, *Neosieulus longispinosus* revealed that, only higher concentration of 0.7 per cent caused mortality of egg, recording 100 per cent. However, all the concentrations caused 100 per cent mortality of adult, thus not safe for *N. longispinosus.*

- Safety of the methanol fraction at 0.3, 0.5 and 0.7 per cent concentration was evaluated for compatibility with the acaropathogen, *Acremonium zeylanicum* by poisoned food technique. Even the lowest concentration was found to cause inhibition of 40.37 per cent. Thus methanol fraction can be considered incompatible to *A. zeylanicum.*

- The study identified the potential acaricidal action of the crude extracts of the botanicals *Acorus calamus*, *Carica papaya*, *Annona squamosa*, *Bacopa monnieri*, *Aegle marmelos* and *Lantana camara* against the spider mite, *T. truncatus*. The methanol fraction of *A. calamus* emerged as a promising candidate in mite pest management under protected cultivation.

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BIOEFFICACY OF BOTANICALS AGAINST THE SPIDER MITE, *TETRANYCHUS TRUNCATUS* EHARA (PROSTIGMATA: TETRANYCHIDAE)

by

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ABSTRACT OF THE THESIS

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Abstract

The spider mite, *Tetranychus truncatus* Ehara is a polyphagous mite pest infesting many economically important crops of Kerala. Synthetic acaricides, though effective in mite management, pose several adverse effects to human and environment, demanding a shift to safer and easily biodegradable products. Exploitation of botanicals can be a viable alternative to synthetic acaricides to overcome these hazards. However, botanicals have not been evaluated and effectively utilized for mite pest management in Kerala.

The study entitled "Bioefficacy of botanicals against the spider mite, *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae)" was carried out at Acarology Laboratory, Department of Agricultural Entomology, College of Horticulture, Vellanikkara during 2018 to 2020. The objectives of the study were to evaluate the efficacy of botanical extracts against *T. truncatus*, to identify bioactive solvent fractions of promising plant extracts against *T. truncatus*, and to test the safety of bioactive fractions to the predatory mite, *Neoseiulus longispinosus* (Evans) and the acaropathogen, *Acremonium zeylanicum* (Petch).

The crude aqueous (5, 7.5 and 10%) and methanol (1 and 2 %) extracts of ten plants *viz*., *Acorus calamus* L., *Bacopa monnieri* (L.) Pennell, *Quassia indica* Gaern., *Eucalyptus* sp., *Lantana camera* L., *Aegle marmelos* (L.) Correa, *Annona squamosa L., Vitex negundo* L., *Carica papaya* L. and *Ocimum sanctum* L. were evaluated at different concentrations for their efficacy against egg and adult of *Tetranychus truncatus* Ehara in laboratory bioassays. The aqueous extract of the botanicals, though did not show any ovicidal action, differed significantly in their adulticidal action. The aqueous seed extract of *A. squamosa* and leaf extract of *C. papaya*, both at 10 per cent concentration recorded highest mortality (98.67%) of adults, five days after treatment. *B. monnieri* (10%) and *A. squamosa* (7.5 and 5%) also recorded mortality on par with these treatments. Appreciable adulticidal activity was also recorded by *B.* monnieri (7.5 and 5 %), *A. calamus* (10%), *C. papaya* (7.5%) and *L. camera* (10%).

The methanol extract of the botanicals were found to be effective against the adults of *T. truncatus*. Cent per cent mortality of adult mites was attained in *A. calamus* (2%) within 48 h of treatment. By 72 h, *C. papaya* (2%) and *A. calamus* (1%) also

recorded 100 per cent mortality. Four days after treatment, *A. marmelos* (2%) and *C. papaya* (1%) also recorded significant mortality of adult mite, on par with these treatments, followed by *A. squamosa* (2%), *B. monnieri* (2%) and *A. marmelos* (1%). However, the methanol extracts did not record appreciable ovicidal activity.

The botanical, *A. calamus*, found effective against the gravid females of *T. truncatus* based on the laboratory studies, was further subjected to bioassay guided fractionation, to identify the bioactive solvent fraction. Hexane, chloroform and methanol were employed as solvents based on the increasing order of polarity. Fractionated methanol extract at 0.7 per cent concentration resulted in 100 per cent mortality of eggs. All the three solvent fractions were promising against adult mites, recording 100 per cent mortality by 96 h.

The efficacy of the methanolic fraction of *A. calamus* (0.3 and 0.5%) and aqueous extract of *A. squamosa* (7.5%) was evaluated along with neem oil emulsion (2%), horticultural mineral oil (HMO 2.5%) and spiromesifen (100 g a.i/ ha) against *T. truncatus* on cucumber, in polyhouse. Within seven days of treatment, 0.5 and 0.3 per cent *A. calamus* recorded significant per cent reduction in mite population closely following the synthetic acaricide, spiromesifen. By tenth day, *A. calamus* (0.5%) and spiromesifen recorded complete reduction in mite population. The lower concentration of 0.3 per cent also recorded mite population on par with these. These treatments were followed by *A. squamosa* and HMO, in reducing the mite population.

Laboratory bioassay to evaluate the safety of the methanolic fraction of *A. calamus* to the predatory mite, *N. longispinosus* resulted in 100 per cent mortality of the adult. Evaluation of safety of the methanolic fraction of *A. calamus* to the acaropathogen, *A. zeylanicum* using poisoned food technique revealed antagonistic effect on the acaropathogen. The methanolic fraction caused 62.96, 54.07 and 40.37 per cent inhibition at concentrations of 0.7, 0.5 and 0.3 per cent, respectively.

The study identified appreciable acaricidal property of the botanicals *A. calamus*, *C. papaya, B. monnieri*, *A. squamosa, A. marmelos* and *L. camera* against spidermites. The methanolic fraction of *A. calamus* emerged as a promising candidate in mite pest management under protected cultivation.