BIOEFFICACY OF *Tagetes minuta* L. AGAINST *Tetranychus truncatus* EHARA (PROSTIGMATA: TETRANYCHIDAE) AND *Aphis craccivora* KOCH (HEMIPTERA: APHIDIDAE)

By

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(2020-11-039)

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KERALA, INDIA
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THESIS

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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
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2023

DECLARATION

I, hereby declare that the thesis entitled "Bioefficacy of *Tagetes minuta* L. against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae) and *Aphis craccivora* Koch (Hemiptera: Aphididae)" 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 *Tagetes minuta* L. against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae) and *Aphis craccivora* Koch (Hemiptera: Aphididae)" is a record of research work done independently by Mr. Ashish. V. V. 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 him.

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Introduction

1. INTRODUCTION

Synthetic pesticides are indispensable in intensive agricultural production, serving as backbone in insect pest management. However, extensive use of synthetic pesticides over decades has led to several adverse effects on environment and non-target organisms. Hence, there has been a growing need for alternative, environmentally benign, effective and biodegradable pesticides with greater selectivity. This has generated interest in the development of biopesticides based on botanicals. Botanicals offer an attractive and favourable alternative for synthetic pesticides due to their rapid degradation, target specific nature and less phytotoxicity (Vasquez *et al*., 2016).

Tagetes minuta L., commonly known as Mexican marigold, wild marigold and southern cone marigold an annual herb that belongs to the family, Asteraceae. The biological activities of the plant have been documented worldwide. Insecticidal properties of *T. minuta* were documented against several pests of field crops, stored products and public health (Perich *et al*., 1995; Shahzadi *et al*., 2010; Phoofolo *et al*., 2013). The plant is also known to possess acaricidal property against mite pests (Mmbone *et al*., 2014). However, very few studies have been conducted in India, particularly in Kerala to evaluate the insecticidal and acaricidal properties of this plant.

Spider mites of the family Tetranychidae are important phytophagous pests that attack a variety of crops, ornamental plants, and medicinal plants worldwide. They have the potential to cause significant yield losses (Roy *et al*., 2011). Spider mites are significant pests of vegetables in India and have been observed to reduce yields by 7 to 48 per cent. (Srinivasa and Sugeetha, 1999). The mites colonise the lower surface of leaves, close to the midrib and veins. However, during a severe infestation, they can also be seen on the upper surface of leaves and on twigs. The first sign of red spider mite infestation is the chlorotic and stippled appearance of the leaves due to loss of chlorophyll, leading to slower photosynthesis. Leaves that have been severely affected become entirely pale, deform, dry out, and drop off. The spider mite species, *Tetranychus truncatus* Ehara dominates the vegetable crops of Kerala (Bennur *et al*., 2015).

The cowpea aphid, *Aphis craccivora* (Hemiptera: Aphididae) is an important sucking pest of leguminous crops throughout India. It feeds on aerial parts by sucking and extracting plant sap, and decreases the quantity of nutrients and water available to the crop leading to significant yield loss (Jagdish *et al.*, 2011). When the infestation is severe, especially at the seedling stage, the aphid causes symptoms like chlorosis and stunting that delay the commencement of blooming and even leads to plant mortality. It also causes indirect damage to the plant by excreting copious amount of honey dew, which leads to the development of sooty mould on surface of leaves, thereby reducing the rate of photosynthesis. Besides, they are also vectors of important viral diseases affecting the legumes.

Farmers depend on synthetic pesticides for the management of mite and aphid pests on vegetables. Novel synthetic acaricides now being extensively used for mite management in the vegetable crops in Kerala include spiromesifen, fenazaquin, diafenthiuron and fenpyroximate, due to their rapid action. Similarly, the popular insecticides used against aphids include neonicotinoids like imidacloprid, acetamiprid and thiamethoxam, in addition to the conventional systemic insecticides. The excessive and indiscriminate use of synthetic pesticides for their management has, however, led to the development of resistance in the populations of mites and aphids. Recent reports indicate that *T. truncatus* populations in vegetable crops in Thrissur, Kerala has become significantly resistant to three regularly used new acaricides *viz*., spiromesifen, fenazaquin, and diafenthiuron (Bacchar *et al*., 2019). Similarly, *A. craccivora* has been reported to exhibit considerable degree of resistance to newer insecticides like imidacloprid (Dawood and Farghaly, 2016) and dinotefuran (Mokbel and Mohamed, 2009), among many others.

In the above context, these two pest species *viz*., *T. truncatus* and *A. craccivora* were found to be the ideal pests for evaluation of pesticidal effects of the botanical *Tagetes minuta*, under Kerala conditions. The study entitled “Bioefficacy of *Tagetes minuta* L. against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae) and *Aphis craccivora* Koch (Hemiptera: Aphididae)” was conducted to identify the acaricidal and insecticidal effects of *T. minuta* extracts for pest management.

The objectives of the study include:

1. To identify the bioactive solvent fractions of *Tagetes minuta* L.

2. To evaluate the bioefficacy of the solvent fractions against *Tetranychus truncatus* and *Aphis craccivora.*

Review of literature

2. REVIEW OF LITERATURE

In recent years, crop protection measures against economically important insect pests have heavily relied on plant products. Numerous studies have shown the toxicity of several plant extracts, but only a small number of them are offered commercially in the agricultural sector. Several studies have been made on the pesticidal properties of the Mexican marigold, *Tagetus minuta*. This chapter reviews the bioactive properties of *T. minuta* and its related species with special emphasis on the acaricidal and insecticidal properties. Since limited number of studies were being conducted to assess the acaricidal and insecticidal properties of this botanical, literature on evaluation of other botanicals against insect and mite pests is also reviewed here.

2.1. Taxonomy and origin of *Tagetes minuta* L.

The genus *Tagetes*, which has about 23,000 species and over 1,000 genera, is one of the most abundant plant taxonomic groups. *Tagetes minuta* L., an annual aromatic herb, is a member of the family Asteraceae (Sadia *et al*., 2013). It has numerous common names, including Mexican marigold, wild marigold, stinkweed, smelling roger and khaki bush (Randall and Kessal, 2004). Argentina, Chile, Bolivia and Peru are just a few of the nations in southern South America where the plant is indigenous to temperate grasslands and mountainous regions (Soule, 1993). Since its introduction, the plant has spread to many locations throughout the world, including Africa, Australia, New Zealand, the United States, Europe, and Asia, where it thrives freely as a weed despite being recently produced commercially in many places (Naqinzhad and Mehrvarz, 2007).

2.2. Morphology and habit of *Tagetes minuta* L.

Tagetes minuta is an annual herb that grows 50-150 cm tall and has an upright, occasionally highly branched stem (Shahzadi, 2012). The 3-30 cm long and 0.7-8 cm broad leaves are typically opposing but frequently alternate in the top regions of the plant. The leaves are aromatic, glabrous, pinnately compound with 9-17 leaflets, dark green to somewhat glossy green and pungent. The leaflets have linear-lanceolate margins that are delicately serrulate, with orange transparent glands and are 2-4 cm

long. The leaf margins are sharp and serrate. Small, multicellular, punctate glands, which are orangish in colour and emit a liquorice-like scent when punctured, are present on the underside of the leaves (Prakas Rao, 1999).

The cymes, which are typically flat-topped, yellowish green in colour, 10 to 15 mm long, and 2-3 mm wide with each being enclosed by four or five joined involucre bracts, support the many heads. Per capitula, there are typically 3 to 5 yellow-orange disc florets and 10 to 15 yellow-orange ray florets. The achenes are cylindrical, dark brown to black in colour, 10 to 12 mm long and have a pappus made up of 3-5 elliptical to lanceolate scales that are 0.5-1 mm long and 1-4 small, uneven awn-like scales that are 2-3 mm long.

2.3. Chemical profile of *Tagetes minuta* L.

Tagetes minuta's essential oils and extracts have been thoroughly examined for their various bioactivity properties both *in vivo* and *in vitro*, and they have been found to have antimicrobial, insecticidal, nematicidal, acaricidal and repellent effects against a variety of human, livestock and plant pests and pathogens. The chemical makeup of *T. minuta* essential oils obtained from various parts of the world has been investigated and compared. Numerous environmental and anthropogenic factors have been implicated in the reported significant variances in the chemical profiles of *T. minuta* essential oils.

Several *Tagetes* species and chemotypes have been recognized as sources of various kinds of physiologically active secondary metabolites that have pesticidal and medicinal value. According to Morallo-Rejesus and Decena (1982), the weed *T. minuta* contains the pesticide-like compounds 5-(3-buten-1-ynyl)-8, 2-bithienyl and alpha therthienyl. It has been claimed that alpha terthienyl, in its synthesised form, is the active ingredient in marigolds (Kanagy and Kaya, 1996; Olabiyi and Oyedunmade, 2007).

The marigold tissues contain a number of chemicals that are biologically active against a variety of species (Vasudevan *et al*., 1997). Thiophene, one such compound, has nematicidal, insecticidal, antiviral, antibacterial and antifungal effects (Marles *et al*., 1992, Margl *et al*., 2002, Riga *et al*., 2005). Depending on the marigold species, the

stage of the plant's development and the vegetative organ, different marigold plants have different thiophene concentrations (Margl *et al*., 2002; Gil *et al*., 2002). For instance, Jacob *et al*. (1994) reported that *Tagetes patula* L. has higher thiophene contents than *Tagetes erecta* L. The highest diversity and concentrations of thiophene is found in marigold roots (Tosi *et al.,* 1991), and their concentration levels rise with the age of the plant, peaking during the reproductive phases (Gil *et al*., 2002).

The variations in the chemical composition of extracts or essential oils from the same plant species result in chemotypes, which are the result of biological variations caused by the effects of different soils, temperature, weather conditions and light among other factors. This implies that it is possible for the chemical composition of plants that are botanically identical to vary. For example, a comparison of the chemical composition of *T. minuta* essential oils from Madagascar showed that the (Z)-β-ocimene and dihydrotagetone constituents were considerably lower than those of *T. minuta* essential oils from India, Turkey, Rwanda and France (Ramaroson-Raonizafinimanana *et al*., 2009).

Chemotypic variation of *T. minuta* essential oils has been reported in several studies. For instance, untargeted analysis of GC-MS data and hierarchical clustering analysis (HCA) of *T. minuta* essential oils from South Africa has revealed two major chemotypes (Tankeu *et al*., 2013).

2.4. Bioactive properties of *T. minuta*

Because of the plant's distinctive chemical makeup and bioactivities, Mexican marigold and its derivatives have a long history of being used by humans for food, medicine and aromatherapy. Essential oils of *T. minuta* have attracted a lot of attention in recent years, and several studies have been conducted on their phytochemistry, bioactivities and applications. The rising consumer demand for natural products like additives, medicines and insecticides, whose global acceptability and safety are highly valued in comparison to synthetic items, is partly to blame for the interest in essential oils.

A wide range of plant, human, and animal pathogens, pests, and parasites are susceptible to the bioactivities and therapeutic properties of *T. minuta* essential oils,

including: antihelminthic, carminative, arthropod repellency, sedative, weedicidal, antiseptic, diaphoretic, spasmolytic, germicides, stomachic, antispasmodic, antiprotozoal and bactericidal. *T. minuta* is therefore a potentially valuable agent for safeguarding animals as well as food crops both on the farm and in storage thereby promoting food security and improving human livelihoods. However, in order to position this plant as a new generation crop globally, increased value addition and the requirement for validating historically asserted uses and applications of *T. minuta* essential oils through extensive scientific investigations should be prioritised (Gakuubi *et al*., 2016).

2.4.1. Antibacterial properties of *Tagetes minuta*

One of the most extensively researched aspects of essential oil research is antibacterial activity. *T. minuta* essential oils have been shown to have antibacterial activity against a variety of pathogenic microorganisms that affect people, plants, and animals. Muyima *et al*. (2004) evaluated the antibacterial and antioxidant properties of the essential oils from three South African plants, including *T. minuta*. The antibacterial properties of essential oils against two Gram-positive bacteria, *Bacillus subtilis* (Ehrenberg) Cohn and *Staphylococcus aureus* Rosenbach, and two Gram-negative bacteria, *Escherichia coli* (Migula) Castellani and Chalmers and *Pseudomonas aeruginosa* (Schroeter) Migula, were assessed using agar diffusion assays. When used against the test bacterium, *T. minuta* essential oils containing the main ingredients (Z)-ocimene and dihydrotagetone showed concentration-dependent antibacterial activity, with an increase in activity with concentration.

Although Gram-positive bacteria were more susceptible to the effects of the essential oils than Gram-negative bacteria, this result was in agreement with earlier research that found Gram-negative bacteria to be more resistant to the effects of essential oils than Gram-positive bacteria (Trombetta *et al*., 2005).

In a different study, *T. minuta* essential oils' chemical makeup, antioxidant, antibacterial, and cytotoxic properties were assessed. Two Gram-negative bacteria, *Salmonella typhi* (Schroeter) Warren & Scott and *E. coli*, as well as two Gram-positive bacteria, *S. aureus* and *B. subtilis*, were used to test the essential oils' antimicrobial

effects. Additionally, the minimum inhibitory concentrations (MICs) of the essential oil for the test pathogens were determined using the microdilution method. The primary components of *T. minuta* essential oils, which included dihydrotagetone, (E)-ocimene, tagetone, (Z)-ocimene, limonene, and epoxyocimene, had concentration dependent bactericidal activity. The MICs were 150 + 8, 165 + 9, 67 + 8, and 75 + 7 g/mL of *T. minuta* EOs for *S. typhi, E. coli, S. aureus,* and *B. subtilis*, respectively (Shirazi *et al*., 2014).

The antibacterial effectiveness of *T. minuta* essential oil with various chemical profiles and geographic origins has also been investigated. The antibacterial activity of essential oils extracted from aerial parts of *T. minuta* collected from Egypt, South Africa and the United Kingdom was evaluated against eight bacterial pathogens. Gram-positive bacteria (*B. cereus, B. subtilis, S. aureus* and *S. faecalis*) were found to be more resistant to the inhibitory effects of essential oils than Gram-negative bacteria (*Proteus mirabilis* Hauser, *P. aeruginosa, E. coli* and *S. typhi*) (Senatore *et al*., 2004).

2.4.2. Antifungal properties of *Tagetes minuta*

There have been reports of *T. minuta* essential oils and plant extracts having antifungal action against a variety of pathogenic fungi. In the study, among the 49 essential oils examined, limonene, 1,8-cineole, pinene, pinene, myrcene and camphor were found to be the most frequently occurring components in oils exhibiting high levels of antifungal activity. These compounds have been identified as some of the common components of *T. minuta* essential oils. Numerous fungi, including *Rhizoctonia solani* Kuhn, *Fusarium oxysporum* Schltdl, *Penicillium digitatum* (Pers.) Sacc., *Aspergillus niger* Tieghem, *Verticillium fungicola* (Preuss) Hassebr., and *Trichoderma harzianum* Rifai have been demonstrated to be sensitive to limonene, 1,8-cineole, pinene, and camphor (Marei *et al*., 2012).

Essential oils from *Tagetes minuta* have been tested for their fungicidal abilities against a variety of fungi. With regard to eight phytopathogenic fungi, specifically *Rhizoctonia solani, Fusarium solani* (Mart.) Sacc., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Fusarium oxysporum pisi, Sclerotium rolfsii* Sacc., *Pyricularia grisea* (Cooke) Sacc., *Fusarium oxysporum lentis* and *Alternaria solani* Sorauer, essential oils from *T.*

minuta's leaves were more active than oils from the plant's flowers. The range of per cent inhibition by the essential oils from flowers at 1000 g mL-1 was 8.9 to 35.1 per cent, with the highest activity found against *F. oxysporum pisi* and the lowest activity against *P. grisea*. As opposed to this, leaf essential oils displayed varying degrees of action against *S. rolfsii* and *F. oxysporum lentis* (Supradip *et al*., 2012).

Essential oils from *T. minuta*'s aerial parts were found to have concentration-dependent antifungal activity against *Aspergillus niger* Tieghem and *Candida albicans* (Robin) Berkhout, with minimum inhibitory concentration (MIC) values of 135-15 and 115-8 g/mL, respectively (Shirazi *et al*., 2014).

2.4.3. Nematicidal properties of *Tagetes minuta*

Two chemicals, (Z)-ocimene and dihydrotagetone obtained from *T. minuta*, were tested for toxicity against the eggs and juveniles of the plant-parasitic nematode, *Meloidogyne incognita* (Kofoid & White). Essential oils from *T. minuta* were very harmful to the test nematode's eggs and juveniles at concentrations of 4, 3, 2 and 1 per cent. Additionally, the aforesaid oil concentrations inhibited egg hatching by 72 to 79 per cent over a period of 14 days, with the inhibitory action being concentration-dependent. Dihydrotagetone was more toxic to *M. incognita* eggs than (Z)-ocimene, according to the study, and it killed juveniles considerably faster (in 72 hours) than (Z)-ocimene did (in 96 hours) (Adekunle *et al*., 2007).

Tagetes minuta essential oils have been researched for their nematicidal activity against animal gastrointestinal nematodes in addition to plant-parasitic nematodes, such as *Haemonchus contortus* (Rudolphi), a significant pathogenic nematode of ruminants. Two bioassays were employed in the study: the larval development test (LDT) and the egg hatch test (EHT). At a dosage of 2.5 mg mL^{-1}, *T. minuta* essential oils inhibited 98.1 per cent of *H. contortus* larvae from hatching in the EHT. Additionally, 0.53 mg mL^{-1} was the effective essential oils dose (EC_{50}) that stopped 50 per cent of egg hatching. In the test for larval development, *T. minuta* essential oil inhibited 99.5 per cent of the growth of *H. contortus* at a dose of 10 mg mL^{-1}, with an EC_{50} value of 1.67 mg mL^{-1} (Macedo *et al*., 2013)

2.4.4. Bioefficacy of *T. minuta* against insects

Tagetes minuta extracts were discovered to have a considerable impact on the mortality, fertility, and hatchability of various insect pests. These findings can be effectively used in pest management strategies.

Studies were carried out by Weaver *et al*. (1994) to ascertain the potency and toxicity of *T. minuta* extracts against *Zabrotes subfasciatus* (Boheman), the Mexican bean weevil. For the floral, foliar and root extracts of *T. minuta*, LC_{50} values for male and female weevils were calculated. The males exposed to the root extract had the highest 24-h LC_{50} values, at 138 g/cm^2, while the females subjected to the foliar extract had the highest values, at 803 g/cm^2 (least susceptible). All LC_{50} values were reduced by 20-30 g/cm^2 when the exposure time was extended to 48 h. Compared to females, males were more vulnerable. In contrast to the root extract data, which suggested slower acting components, the time for incapacitation for 50 per cent of the test insects (IT_{50}) for floral and foliar extracts indicated fast acting, volatile components, likely as a result of the interaction between photophase and time-dependent efficacy. This demonstrated that *T. minuta* floral and foliar extracts may be effective insecticides for managing pests in stored goods.

In a study by Tyagi *et al.* (1997), *T. minuta* essential oil was found to have promising insect repellent properties against three mosquito species *viz*., *Aedes aegypti* (Linnaeus), *Culex quinquefasciatus* Say and *Anopheles stephensi* Liston. After a 6-hour study period, the test oils provided 86.4, 84.2, and 75 per cent protection against *A. stephensi, C. quinquefasciatus* and *A. aegypti* respectively.

The effectiveness of 41 essential oils, including those from *T. minuta*, in deterring the three species of mosquitoes mentioned above was studied. The time between applying a repellent and the first two bites, or the first two bites in consecutive observations, was used to compute the protection time. Essential oils from *T. minuta* provided 480 minutes of protection against *A. stephensi* and *C. quinquefasciatus* and 60 minutes of protection against *A. aegypti* (Amer and Mehlhorn, 2006).

The effects of crude marigold extracts from Mexico (*T. minuta*) on the banana weevil *Cosmopolites sordidus* Germar were investigated. In the laboratory, the impact on mortality, settling reactions, and oviposition was assessed. When compared to controls, oviposition was noticeably lower on corms treated with the extract. The results indicated that though the botanical possesses limited insecticidal properties against the weevil, it has the potentials to control the weevil through preventing oviposition (Tinzaara *et al*., 2006).

Ali *et al*. (2010) assessed the bio-efficacy of four plant leaf extracts against the mustard aphid, *Lipaphis erysimi* (Kaltenbach), on Indian mustard, *Brassica juncea* (L.) Czern. These extracts were *Calotropis procera* (Aiton), *Argemone mexicana* L., *Tagetes minuta* and *Azadirachta indica* A. Juss. The highest percentage reduction of aphids during the first, second and third sprays were at 28.79, 40.52 and 59.32 g/ml; 34.70, 44.49 and 66.14 g/ml; and 53.88, 64.84 and 100.00 g/ml respectively. Mexican marigold, however, reduced *L. erysimi* by 96.38 percent at the greatest concentration (1: 2.5 g/ml). All of the treatments using plant leaf extracts had insecticidal action, however Indian neem and Mexican marigold significantly decreased the aphid population.

Dunkel *et al*. (2010) studied the interactions of a biopesticide formulation containing a steam-distilled shoot extract of Mexican marigold, *T. minuta*, and entomopathogenic fungus to manage the sugarbeet root maggot, *Tetanops myopaeformis* Roder. To test the claim that this fungicidal and nematicidal biopesticide induces dose-dependent mortality and developmental arrest in *T. myopaeformis*, but does not obstruct the activity of entomopathogenic fungi when employed simultaneously. Shoot extract plus surfactant (*T. minuta* oil) was used in a 65:35 ratio. To test the theory, a soil-Petri dish bioassay system was created. 0.75 per cent *T. minuta* oil treatment (0.458 % active ingredient) was lethal for 93 per cent of the test insects, however 0.5 per cent *T. minuta* oil treatment (0.325 % active ingredient) was sufficient to inhibit pupation without death for diapausing, nonfeeding, but active third-instar larvae.

The n-hexane and ether extracts of *T. minuta* seeds were tested against three prevalent grain pest species *viz.*, *Tribolium castaneum* Herbst, *Rhyzopertha dominica* (Fabricius) and *Callosobruchus analis* (F.). Both extracts showed appreciable insecticidal activity (70%) against the common grain pests without any phytotoxic activity (Shahzadi *et al*,. 2010).

At three hot spots in southern Ethiopia, studies on the impact of wood ash, *T. minuta* extract and hot water treatment on the occurrence enset root mealy bug, *Cataenococcus ensete* Williams and Matile-Ferrero, were conducted. The study was carried out during the agricultural seasons of 2004 and 2006. The results showed that before planting, hot water treatment killed 100 per cent of mealybugs on the contaminated suckers as effectively as chemical pesticide, but subsequent hot water drenching did not completely eradicate them. For the first three months after the application of wood ash and *T. minuta*, the mealybugs' development was slowed, but after that, an increase in population was seen. The chemically treated plot, followed by the hot water treatment, produced the highest pseudostem circumferences and plant height measurements, whereas the untreated plot produced the lowest measurements. According to the study's findings, hot water treatment helped to control the pest's spread and lower the original population of mealybugs. *T. minuta* could be incorporated into the integrated pest management programme to manage the pest in order to reduce the root shocking effect of wood ash (Gemu *et al*., 2010).

In a soil-based bioassay with larval sugarbeet root maggot, *Tetanops myopaeformis* Roder, the effect of *T. minuta* oil on fungal efficacy under concurrent use was investigated using a model system of two entomopathogenic fungi, *Beauveria bassiana* (Bals.) Vuillemin. TM28 and *Metarhizium anisopliae* variety *anisopliae* (Metsch.) Sorokin MA 1200. There were no negative effects of *T. minuta* oil and no evidence of synergy with entomopathogenic fungi; rather, *T. minuta* oil and each fungal isolate separately had additive effects (Dunkel *et al*., 2010).

The ability of *T. minuta* essential oils to repel other insects has also been researched. It was discovered that *T. minuta* essential oils with the primary constituents (Z)-tagetone, (E)-ocimene and dihydrotagetone have effective *Triatoma infestans*

repellant properties (the insect vector of Chagas disease). The essential oils of *T. minuta* produced an average repellency of 94.7 per cent at a concentration of 0.5 per cent (w/v) (López *et al*., 2011).

Tagetes minuta crude extracts were tested against the cabbage aphid *Brevicoryne brassicae* (Linnaeus) for their aphidicidal properties. *T. minuta* crude extracts from acetone, methanol, water, and a mixture of acetone/methanol/water were compared for their fatal and sub-lethal effects (7:7:1, v:v). The mixture, followed by methanol and water as the solvent systems, produced the most toxic extract, while acetone produced the least harmful extract. The linear regression equations that demonstrated a substantial decrease in fecundity as the concentration of crude extracts increased were effective at describing the sub-lethal effect on cabbage aphid reproduction. The mixed solvent system extract had the most impact on fecundity (Phoofolo *et al*., 2013).

Muzemu *et al*. (2013) evaluated the effectiveness of botanical leaf powder from *Eucalyptus tereticornis* Sm., *T. minuta* and *Carica papaya* L. at rates of 5 g, 10 g, and 20 g per 200 g of open pollinated maize grain in controlling *Sitophilus zeamais* Motschulsky (variety ZM421). Twelve treatments were replicated three times in the completely randomised design (CRD) of the trial. Before the botanicals were added, the grains were placed in a freezer at -4°C for a fortnight to destroy any weevil inoculum and eggs that may have already been present in the grain. In 750 ml jars, 200 g of maize grains were contaminated with 200 three-week-old, unsexed pure culture weevils. Weevils were sieved at 14, 28, 42, 56, and 70 days to determine their mortality. The percentage of grain weight loss was measured 35 days after the introduction of the pests. The results showed that 56 hours after treatment, *T. minuta* leaf powder at a dosage of 20g/200g grain induced 79.44 percent mortality of the weevils.

Wanzala and Ogoma (2013) used the human-bait method to study the chemical makeup and repellent effects of *T. minuta* essential oil on the host-seeking female *Anopheles arabiensis* Patton mosquitoes. The essential oil of *T. minuta*, which contained the major ingredients (Z)-ocimene, (E)-ocimene, (Z)-tagetone, (E)-tagetone, dihydrotagetone and piperitenone, had a considerably greater dose-response effect on repellency than the control arm treated with pure petroleum jelly.

Tagetes minuta extracts were evaluated for their efficacy and repellency against black bean aphids (*Aphis fabae* Scopoli) in the laboratory. Mortality potential of the plant was determined by spraying the crude extracts on the pests reared earlier in the screen house. *T. minuta* crude extracts at concentrations of 50 g/L, 100 g/L, 150 g/L soaked for 24 hours and also 150 g/L soaked 48 hours in distilled water. Treatment using *T. minuta* at concentration 150 g/L soaked for 48 hours caused the highest mortality in aphids (Mmbone *et al*., 2014).

Tagetes minuta and a number of other plants growing in the lower Himalayan regions were tested at 2 per cent concentration, for their ability to repel the peach fruit fly *Bactrocera zonata* Saunders (Diptera: Tephritidae). Maximum mortality of male fruit fly due to *T. minuta* extract was 73.00 per cent. In comparison to the 62.60 per cent pupae recovered from untreated guavas, the lowest number of pupae, 3.30 per cent, were found in guavas treated with *T. minuta* extract. Compared to 45.3 per cent adults from untreated guavas, the lowest percentage of adults (0.33 %) were recovered from guavas treated with *T. minuta* extract and it had the highest pupae inhibition (94.6 %) and highest level of adult emergence inhibition (99.2 %). This revealed that *T. minuta* can be exploited as a potent source of pesticide against fruit fly, *B. zonata*, due to maximum pesticidal potential as compared to all other plant extracts applied (Khan *et al.*, 2016).

Mexican marigold, garlic, ginger and chilli pepper aqueous extracts were tested for their ability to repel green pea aphids (*Acrythosiphon pisum*). The results of Analysis of Variance (ANOVA) for the after-spray aphid count showed a significant difference between the treatments. The untreated control had the most aphids per plant (seven), whereas the Mexican marigold, ginger and chilli-treated plots had the fewest (zero). The maximum grain yield (2511.3 kg/ha) and the lowest grain yield (1846 kg/ha), respectively, were achieved from Mexican marigold and chilli-treated plots. This finding showed that Mexican marigold was the best natural pesticide for controlling pea aphids (Kora and Teshome, 2016).

The effectiveness of *Cymbopogon citratus* (DC.) Stapf and *T. minuta* essential oils against the sand fly *Phlebotomus duboscqi* Neveu-Lemaire was evaluated by

Nyamwamu (2017). The inner surface and bottom of a sterile pot were coated with 1.0 ml of each essential oil at concentrations of 0.125, 0.250, 0.500, 0.750 and 1mg/ml, as well as the controls, DEET and Tween 80, and thirty adult *P. duboscqi* were aspirated into the pots. Insect mortalities after 24, 48, and 72 hours, as well as the amount of eggs collected from females subjected to the various treatments, were recorded. The results demonstrated that *T. minuta* and *C. citratus* oils were both extremely effective against *P. duboscqi* sandflies with mortality rates of 100.00 and 82.22 percent on female sandflies and 100.00 and 88.89 percent on male sandflies, respectively, after 72 hours of treatment. *C. citratus* was substantially more effective (P=0.05) and killed more male and female sandflies than *T. minuta*. At 24 h, 48 h, and 72 h, there was no statistically significant difference in the mortality rates of males and females exposed to either of the two oils. Compared to female sandflies treated with *C. citratus* oil, those treated with *T. minuta* oil showed much lower mortality rates.

Arena *et al*. (2018) studied the chemical makeup of *T. minuta* essential oils and assessed the contact toxicity of the oils alone and in combination with cypermethrin against adults of the darkling beetle *Alphitobius diaperinus* Panzer. Dihydrotagetone, cis-ocimenone, trans-tagetone and trans-ocimene were the four primary ingredients of the *T. minuta* oil. When used alone, cypermethrin was only marginally harmful to the insect, whereas the essential oils showed a high contact activity. When used in conjunction with essential oils at low concentrations, cypermethrin dramatically boosted the insecticide's toxicity.

The effect of volatiles from cowpea flowers and two companion plants-lemongrass, *Cymbopogon citratus* and Mexican marigold, *Tagetes minuta*- on the olfactory responses of *Megalurothrips sjostedti* (Trybom) was examined by Diabate *et al*. (2019) using Y-tube olfactometer tests and chemical analysis. The findings showed that the volatiles from *T. minuta*'s vegetative stage were repulsive to female thrips but not to males. The volatiles from two herbal plants contained 54 different chemicals, according to the research. Among the main substances, citral and a 4-component mixture that included dihydrotagetone, (Z)-3-hexenyl acetate, limonene and (Z)—ocimene attracted males but repelled females. These findings demonstrate the capability of *T. minuta* volatiles to deter female *M. sjostedti*.

2.4.5. Bioefficacy of *T. minuta* against ticks and mites

Numerous studies on the biocidal effects of plant essential oils against a wide variety of economically significant arachnids, including ticks and mites, have shown encouraging results.

Eguaras *et al*. (2005) evaluated *T. minuta* essential oils for their biological activity against *Varroa destructor*, an ectoparasitic mite of the *Apis mellifera*. The percentage values of mite mortality sprayed with the essential oil concentrations of 3, 4 and 5 per cent were 64, 72 and 72 per cent, respectively.

Ruffinengo *et al*. (2007) demonstrated considerable acaricidal activity of *T. minuta* essential oil against *V. destructor*, when delivered through pulverisation. Chamorro *et al*. (2011) examined the efficacy of *T. minuta* essential oils against *V. destructor* by using different plant parts from different growth stages. The results of the study showed that for essential oils from leaves of blooming plants, leaves of non-bloomed plants and flowers, the mean percentages of dead mites after six hours of treatment were 97.70, 98.33 and 100.00, respectively.

According to a study by Garcia *et al*. (2012) who evaluated the acaricidal activity of essential oils from leaves and stems of *T. minuta* against several tick species, the essential oils of *T. minuta* had over 90 per cent efficacy against four Brazilian tick species, including *Rhipicephalus microplus, Rhipicephalus sanguineus*, *Amblyomma cajennense* and *Argas miniatus* at a concentration of 20 per cent. This degree of efficacy is comparable to a number of referenced conventional acaricides.

Nchu *et al.* (2012) evaluated the anti-tick properties of the of *T. minuta* essential oil against the tick species *Hyalomma rufipes*. A combination of fresh flowers, leaves and soft stems of the plant was used for extraction of essential oil by hydro-distillation. The adult ticks were found to display a dose repellent response to the essential oil. The repellent EC_{50} of the essential oil was found to be 0.072 mL/mL and 0.070 mL/ for male and female ticks respectively.

Andreotti *et al*. (2013) showed that essential oils from *T. minuta* had high activity against larvae, nymphs and adults of the Asian blue tick, *Rhipicephalus microplus*.

In a laboratory study, the acaricidal properties of *T. minuta* were assessed for their effectiveness and repellency against red spider mite (*Tetranychus urticae* Koch). Spraying the crude extracts on the mites that had previously been raised in the screen house helped scientists establish the plant's potential to cause mortality in the mite. Mite mortality following treatment with *T. minuta* at a concentration of 150 g/L soaked for 48 hours was quite high. In the tests for repellency, *T. minuta* at a concentration of 150 g/L soak for 48 hours had the strongest effect on mites, repelling them by 55 per cent, followed by that soaked for 24 hours, at 12 per cent. (Mmbone *et al*., 2014).

Hincapié *et al*. (2014) studied the acaricidal activity of petroleum ether, ethanol and ethyl acetate extracts of *Tagetes verticillate* against the adult females and nymphs of *T. urticae*. Inhibition of egg-hatching was also recorded. Petroleum ether extract caused significant and increasing mortality through the measuring periods (24, 48 and 72h), reaching mortality peaks of 84.4 per cent (LC_{50}:1000 µg/mL) for nymphs and 68.9 per cent (LC_{50}:5000 µg/mL) for adults. It also caused 78.6 per cent inhibition of egg hatching (LC_{50}:1000 µg/mL).

The ethanolic extract obtained from aerial parts of *T. patula* was tested for efficacy against eggs of *Rhipicephalus sanguineus* (Acari: Ixodidae) by ‘egg hatchability test’. The action on ovary cells of engorged females was also tested by ‘adult immersion test’. Results revealed that the extract effectively inhibited egg hatching by 96.98 per cent (LD_{50} = 6.312 mg/mL). Further, microscopic studies of the structure of the ovaries revealed that after treatment with the extract, the oocytes and pedicel cells had undergone significant morphological changes, which directly interfered with the tick's normal embryogenesis and hindered the development of healthy larvae, thereby breaking the life cycle early on (Politi *et al*., 2015).

In the study by Ozman-Sullivan *et al.* (2018), the adulticidal, ovicidal and repellent properties of *T. minuta*. extracts against *T. urticae* raised on bean (*Phaseolus vulgaris* L.) were evaluated. The *T. minuta* essential oil and hydrolate were evaluated

at seven concentrations (0.09 %, 0.18 %, 0.37 %, 0.75%, 1.5 %, 3 % and 6 %) and three concentrations (1.5 %, 3 % and 6 %) respectively. The newly emerged, copulated females on a bean leaf disc in a Petri dish were sprayed with the treatments. On the first day after treatment, counting of dead mites was started and continued every day for five days. After five days, the live adults were removed and the egg hatching was then noted. Results showed that the efficacy of essential oil ranged from 20 to 54 per cent and that of the hydrolates ranged from 2.27 to 43.47 per cent.

Sabahi *et al*. (2018) examined the toxicity of anethole and essential oils of sweet marigold (*Tagetes lucida*), to the mite *Varroa destructor* and to honey bee workers and larvae. Among the treatments, *T. lucida* oil recorded the highest toxicity to mite larvae (LD_{50}: 9580.7 µg/ml) and the lowest toxicity to adult mites (LC_{50}: 1256.27 µg/ml). It was also the least toxic compound to the worker bees with LD_{50} of 85381 µg/ml.

Premalatha *et al*. (2018), evaluated the efficacy of *Tagetes tenuifolia* Cav. along with nine other botanicals, against *T. urticae* under laboratory conditions, using the leaf disc method. *T. tenuifolia* recorded 85.57 per cent acaricidal activity against the mite at 72 hours after treatment.

Along with other botanicals, leaf extract of *Tagetes erecta* was evaluated for its efficacy against *Petrobia harti* Ewing (Acari: Tetranychidae) infesting medicinal weed, *Oxalis corniculata* L. (Oxalidaceae). Results showed that *T. erecta* leaf extract at 1.5 per cent registered mean mortality of 82.63 per cent and a mean repellency of 70 per cent against the mite (Mitra *et al*., 2021).

Essential oil and aqueous extracts of *T. minuta* leaves along with those of *Azadirachta indica* seed and leaves, respectively and a few chemical acaricides were examined in the lab for their effectiveness against the two-spotted spider mite *T. urticae* on potato (*Solanum tuberosum* L.) in Eastern Hararghe, Ethiopia. Among the botanicals, the *A. indica* seed oil at 5 per cent caused the highest mortality of adult mites (72–100 %) while *T. minuta* leaf oil had higher toxic effects on eggs (0.239 %) (Yigezu *et al*., 2022).

Materials and methods

3. MATERIALS AND METHODS

The present study entitled "Bioefficacy of *Tagetes minuta* L. against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae) and *Aphis craccivora* Koch (Hemiptera: Aphididae)" was conducted in the Acarology laboratory, Department of Agricultural Entomology, College of Agriculture, KAU, Vellanikkara during 2020-2022. The objectives of the study were to identify the bioactive fractions of the plant extract of *Tagetes minuta* and evaluation of the bioefficacy of the fractions against *Tetranychus truncatus* and *Aphis craccivora*. The materials used and methods employed for conducting various experiments based on the objectives set forth in the study are presented herewith.

3.1. Identification of bioactive solvent fraction of *Tagetes minuta* against *Tetranychus truncatus* and *Aphis craccivora*

Tagetes minuta plants cultivated in the Department of Floriculture and Landscaping, College of Agriculture, Vellanikkara (Plate. 1) were used for the bioefficacy studies against *T. truncatus* and *A. craccivora.* The above-ground parts of the plants were harvested at the flowering stage and shade dried in the Insectary building of the Department of Agricultural Entomology (Plate. 2). The dried plants were finely powdered using an electric grinder and stored in air tight containers (Plate. 3) at 4 º C in a refrigerator, until further used.

3.1.1. Extraction of solvent fractions of *Tagetes minuta*

The dried and powdered botanical was extracted sequentially based on the polarity of the solvents into three separate fractions (Plate. 6), using hexane (non-polar), chloroform (medium polar) and water (highly polar) as solvents as per Fig. 1.

Pulverized plant material was weighed and extracted using hexane (3 times of volume) by placing it in rotary shaker for 48 h (Plate. 4). The solution was then filtered and the filtrate was concentrated by a rotary evaporator at 40 º C (Plate. 5) to obtain the hexane fraction. The residue obtained during filtration was re-extracted sequentially using chloroform followed by water, using the same procedure as hexane extraction, to

obtain chloroform fraction and aqueous fraction, respectively (Auamcharoen and Chandrapatya, 2015). These fractions were used for bioefficacy studies against *T. truncatus* and *A. craccivora.*

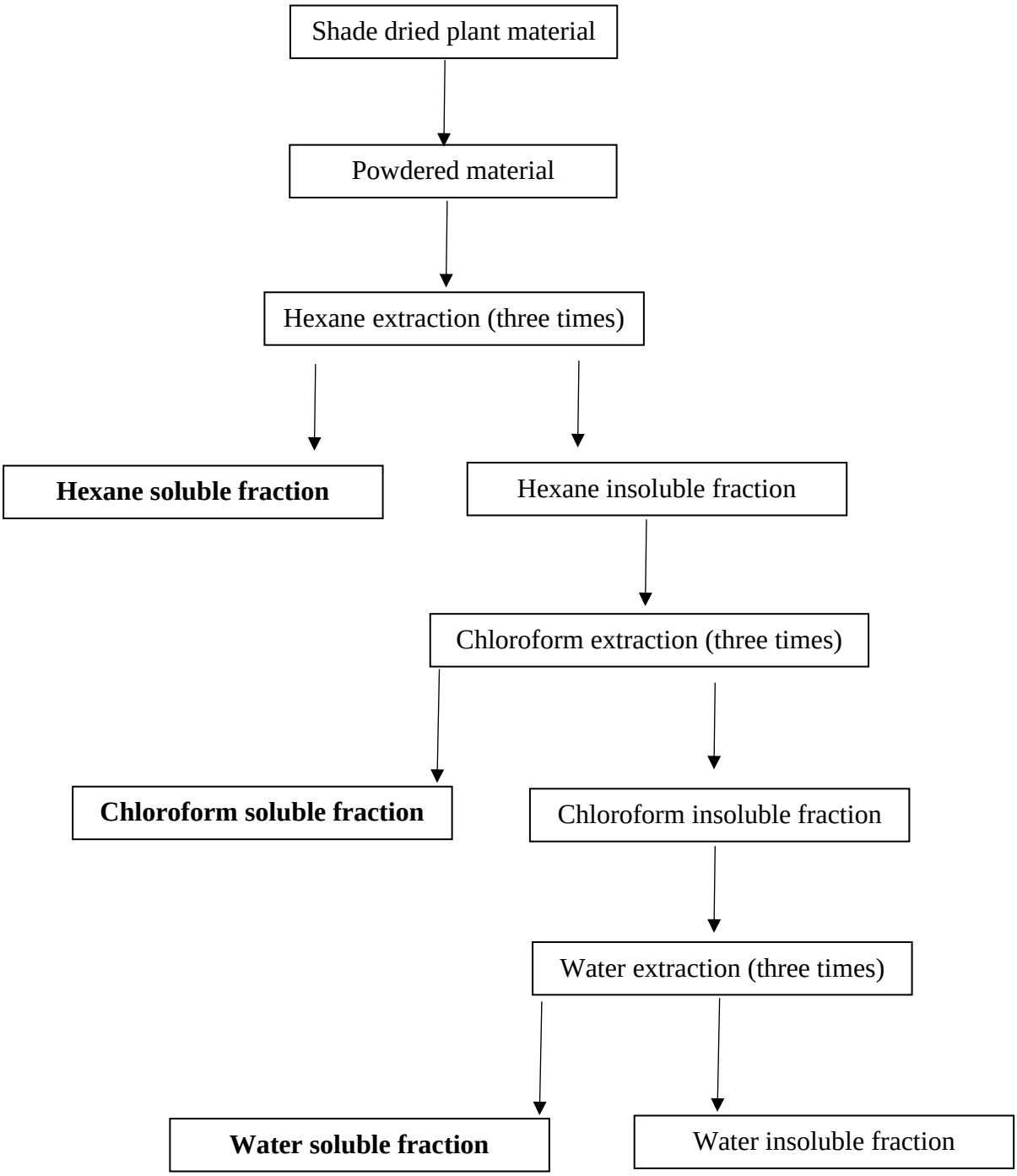

Fig 1. Extraction of solvent fractions from *Tagetes minuta*

Plate 1a. Potted plants of *T. minuta*

Plate 1b. Harvesting of aerial parts

Plate 1. *Tagetes minuta*

Plate 2a. Before drying

Plate 2b. Dried botanical

Plate 2. Air drying of the botanical

Plate 3a. Botanical cut into pieces

Plate 3b. Grinding

Plate 3c. Pulverized botanical

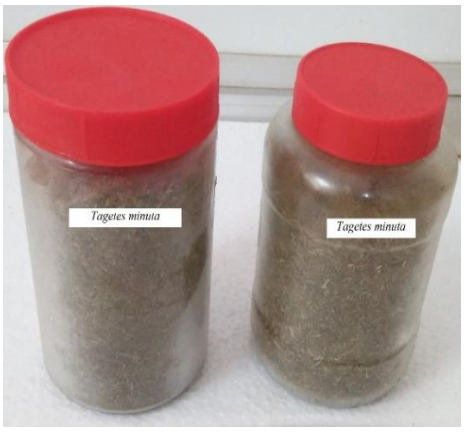

Plate 3d. Storage

Plate 3. Preparation of powdered botanical

Plate 4a. Soaking in solvent

Plate 4b. Shaking in rotary shaker

Plate 4c. Filtration

Plate 4d. Storage

Plate 4. Botanical at various stages of extraction

Plate 5a. Rotary evaporator

Plate 5b. Extract after evaporation of solvent

Plate 5. Solvent evaporation using rotary evaporator

Plate 6a. Hexane fraction

Plate 6b. Chloroform fraction

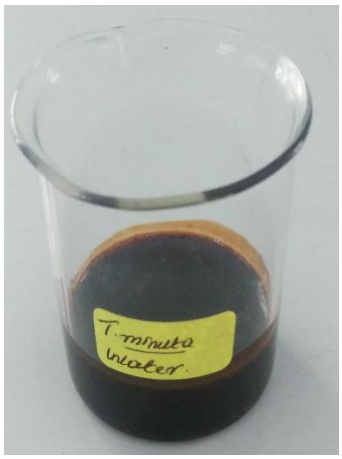

Plate 6c. Aqueous fraction

Plate 6. Solvent fractions of *Tagetes minuta*

3.1.2. Laboratory evaluation of bioefficacy of *Tagetes minuta* solvent fractions

3.1.2.1. Bioefficacy of *Tagetus minuta* solvent fractions against *Tetranychus truncatus*

The solvent fractions of *T. minuta* were evaluated for acaricidal activity against *T. truncatus.* Efficacy of hexane, chloroform and aqueous fractions at five different concentrations (0.025, 0.05, 0.1, 0.15 and 0.2 %) were evaluated separately on eggs and adults of *T. truncatus*.

3.1.2.1.1. Laboratory culture of *T. truncatus*

Tetranychus truncatus was cultured in the laboratory on excised mulberry leaves. Gravid females collected from the nucleus culture of *T. truncatus* maintained in the Acarology laboratory of All India Network Project on Agricultural Acarology (AINPAA) were used to initiate the culture. The females were released onto mulberry leaves, placed upside down on moistened sponge pad kept in plastic tray (40 cm × 28 cm) (Plate.7). The leaves were replaced at an interval of three to four days, by placing the old leaf harboring mites over a fresh leaf, so that the mites got transferred to new leaf naturally, and later the old leaves were removed.

3.1.2.1.2. Ovicidal effect of solvent fractions

The effect of the three solvent fractions of the botanical at five different concentrations (0.025, 0.05, 0.1, 0.15 and 0.2 %) were evaluated against the eggs of *T. truncatus* by following topical application method (Yadav, 2018). The experiment was laid out in Completely Randomised Design with 17 treatments and three replications (Table. 1) (Plate 8).

Mulberry leaf bits (5×5 cm^2) were placed in Petri plates (200×30 mm) lined with moistened cotton. Ten gravid females (from the laboratory culture) 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. The number of eggs per leaf bit were counted under a

stereomicroscope and the eggs in excess of 25 numbers 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 fractions. Eggs on mulberry leaves sprayed with water and emulsifier alone served as control. An absolute control was also maintained without any treatment. The hatchability of eggs after 24, 48, 72, 96 and 120 h of spraying was recorded and per cent mortality of egg was calculated at 120 h.

The data on per cent hatchability at 24, 48, 72, 96 and 120 h and per cent mortality of eggs at 120 h were subjected to analysis of variance (ANOVA) using the software, GRAPES 1.0.0, developed by Kerala Agricultural University. The result obtained was subjected to LSD (Least Significance Difference Test).

Table.1. Experimental design for evaluation of ovicidal effect of solvent fractions on *Tetranychus truncatus*

Design : Completely Randomized Design (CRD)	
No. of treatments : 17	No. of replications : 3
No. of eggs/replication : 25	
T_1 : Hexane fraction (0.025 %)	T_{10} : Chloroform fraction (0.2 %)
T_2 : Hexane fraction (0.05 %)	T_{11} : Aqueous fraction (0.025 %)
T_3 : Hexane fraction (0.1 %)	T_{12} : Aqueous fraction (0.05 %)
T_4 : Hexane fraction (0.15 %)	T_{13} : Aqueous fraction (0.1 %)
T_5 : Hexane fraction (0.2 %)	T_{14} : Aqueous fraction (0.15 %)
T_6 : Chloroform fraction (0.025 %)	T_{15} : Aqueous fraction (0.2 %)
T_7 : Chloroform fraction (0.05 %)	T_{16} : Control (Water + emulsifier)
T_8 : Chloroform fraction (0.1 %)	T_{17} : Absolute control
T_9 : Chloroform fraction (0.15 %)	

Plate 7a. Laboratory culture of *T. truncatus* on mulberry leaves

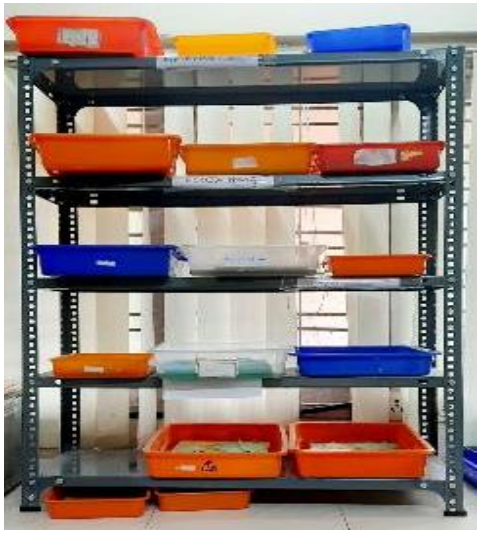

Plate 7b. Mass culture in plastic trays

Plate 7c. Eggs and nymphs of *T. truncatus*

Plate 7d. Adults of *T. truncatus*

Plate 7. Mass culture of *Tetranychus truncatus*

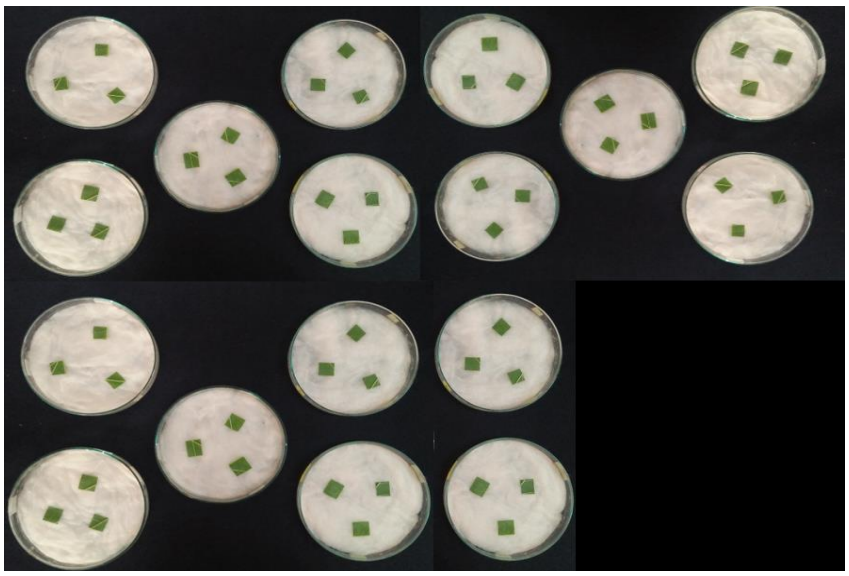

Plate 8. Experimental setup for ovicidal bioassay

Plate 9. Topical application of treatments

3.1.2.1.3. Adulticidal effect of solvent fractions

The effect of the three solvent fractions (0.025, 0.05, 0.1, 0.15 and 0.2 %) of the botanical at five different concentrations was evaluated against the gravid females of *T. truncatus* by topical method (Roy *et al.*, 2015).

Mulberry leaf bits (5×5 cm^2) were placed in Petri plates (200×30 mm) lined with moistened cotton. Twenty-five gravid females (from the laboratory culture) were released per leaf bit. Leaf bits containing *T. truncatus* adults were sprayed with appropriate treatments using a hand atomizer (2ml/bit). Three replications were maintained for each concentration of the fractions. Adult mites on mulberry leaves sprayed with water and emulsifier (Triton X-100) alone served as control. An absolute control was also maintained without any treatment. The experiment was laid out in Completely Randomised Design and three replications were maintained per treatment (Table.2). Mortality of mites was recorded 24, 48, 72, 96 and 120 h after treatment and per cent mortality was calculated. Since, natural mortality was observed in the control treatments, the mortalities were corrected by Abbott's formula as follows:

$$\text{Corrected mortality (\%)} = \frac{(\text{Mortality in control} - \text{Mortality in treatment})}{(100 - \text{Mortality in treatment})} \text{ x } 100$$

(Chil-Núñez *et al*., 2018)

The data on per cent mortality of adults at 24, 48, 72, 96 and 120 h after treatment were subjected to analysis of variance (ANOVA) using the software, GRAPES 1.0.0, developed by Kerala Agricultural University. The result obtained was subjected to LSD (Least Significance Difference Test).

Table.2. Experimental design for evaluation of adulticidal effect of solvent fractions on *Tetranychus truncatus*

Design : Completely Randomized Design (CRD)	
No. of treatments : 17	No. of replications : 3
No. of mites/replication : 25	
T_1 : Hexane fraction (0.025 %)	T_{10} : Chloroform fraction (0.2 %)
T_2 : Hexane fraction (0.05 %)	T_{11} : Aqueous fraction (0.025 %)
T_3 : Hexane fraction (0.1 %)	T_{12} : Aqueous fraction (0.05 %)
T_4 : Hexane fraction (0.15 %)	T_{13} : Aqueous fraction (0.1 %)
T_5 : Hexane fraction (0.2 %)	T_{14} : Aqueous fraction (0.15 %)
T_6 : Chloroform fraction (0.025 %)	T_{15} : Aqueous fraction (0.2 %)
T_7 : Chloroform fraction (0.05 %)	T_{16} : Control (Water + emulsifier)
T_8 : Chloroform fraction (0.1 %)	T_{17} : Absolute control
T_9 : Chloroform fraction (0.15 %)	

3.1.2.2. Bioefficacy of *Tagetus minuta* solvent fractions against *Aphis craccivora*

3.1.2.2.1. Laboratory culture of *Aphis craccivora*

Aphis craccivora culture maintained in the polyhouse of Department of Agricultural Entomology were used to initiate the laboratory culture. These aphids were allowed to infest cowpea seedlings grown in pro trays maintained in rearing cages (Plate. 10). New seedlings were allowed to be infested periodically to maintain the culture. Later, in order to obtain uniform aged adult aphids, a few seedlings from the culture were selected and all adults were removed. On the next day, freshly moulted adults were collected and used for the study.

3.1.2.2.2. Evaluation of botanical fractions against *A. craccivora*

The hexane, chloroform and aqueous fractions of *T. minuta* were evaluated at five different concentrations (0.025, 0.05, 0.1, 0.15 and 0.2 %) for efficacy against *A. craccivora*.

Plate 10. Laboratory culture of *A. craccivora*

Plate 11. Nymphs and adults of *A. craccivora*

Plate 12. Experimental setup for bioassay against *A. craccivora*

Cowpea seeds were sown in individual paper cups (one seed per cup) filled with potting mixture (soil and vermicompost in 1:1 ratio) and the seeds were allowed to sprout and grow. At four leaf stage, adult aphids were released at the rate of 20 per seedling and allowed to settle. The seedlings were then sprayed with appropriate treatments using a hand atomizer (3ml/seedling). Three replications were maintained for each concentration of the fractions. Seedlings sprayed with water and emulsifier alone served as control. An absolute control was also maintained without any treatment. The experiment was laid out in Completely Randomised Design (CRD) with 17 treatments and three replications (Table.3) (Plate.12). Nymphs produced were removed from the experimental seedlings periodically to avoid any experimental error due to population build up. The number of adult aphids on the treated seedlings were recorded at 24, 48, 72, 96 and 120 h of spraying and per cent reduction in aphid count was calculated.

Table.3. Experimental design for laboratory evaluation of solvent fractions against *Aphis craccivora*

Design : Completely Randomized Design (CRD)	
No. of treatments : 17	No. of replications :03
No. of aphid/replication : 20	
T_1 : Hexane fraction (0.025 %)	T_{10} : Chloroform fraction (0.2 %)
T_2 : Hexane fraction (0.05 %)	T_{11} : Aqueous fraction (0.025 %)
T_3 : Hexane fraction (0.1 %)	T_{12} : Aqueous fraction (0.05 %)
T_4 : Hexane fraction (0.15 %)	T_{13} : Aqueous fraction (0.1 %)
T_5 : Hexane fraction (0.2 %)	T_{14} : Aqueous fraction (0.15 %)
T_6 : Chloroform fraction (0.025 %)	T_{15} : Aqueous fraction (0.2 %)
T_7 : Chloroform fraction (0.05 %)	T_{16} : Control (Water + emulsifier)
T_8 : Chloroform fraction (0.1 %)	T_{17} : Absolute control
T_9 : Chloroform fraction (0.15 %)	

The data on per cent reduction of aphid count at 24, 48, 72, 96 and 120 h after treatment were subjected to analysis of variance (ANOVA) using the software,

GRAPES 1.0.0, developed by Kerala Agricultural University. The result obtained was subjected to LSD (Least Significance Difference Test).

3.1.3. Qualitative phytochemical analysis

Qualitative phytochemical analysis of the bio-active solvent fractions, identified during laboratory bio-assays, was performed to determine the predominant phytochemical constituents in the botanical. The hexane and chloroform fractions were subjected to GC-MS/MS analysis at Sophisticated Analytical Instrumentation Facility (SAIF), IIT, Mumbai and the major compounds present in the fractions were recorded.

3.2. Evaluation of selected botanical fractions against *T. truncatus* in pot culture

A pot culture experiment was conducted to evaluate the efficacy of the effective concentrations of the fractions against *T. truncatus* in amaranthus. The experiment was laid out in the open area adjacent to the Insectary building, Department of Agricultural Entomology, during January- March, 2022 (Plate. 13). The seeds of the red amaranthus (variety Arun) collected from the Department of Vegetable Science were sown in polybags for germination. One seedling each, at two leaf stage (one week after sowing), was transplanted in polybags of size 35 cm x 20 cm x 20 cm, filled with potting mixture (soil, coir pith compost and cow dung in 2:1:1 ratio). All cultural practices were carried out as per the Package of Practices Recommendations, KAU (2016). Mites were released on 25 days old amaranthus plants at the rate of 25 active mites per leaf, by stapling mite infested mulberry leaf bit (from laboratory culture) of size 3 cm^2 on three leaves, one each of the top, middle and bottom canopy of the plant (Plate. 14).

One concentration each of the hexane (0.15%), chloroform (0.2%) and aqueous (0.2%) fractions were chosen for evaluation, based on their efficacy in the laboratory studies against egg (3.1.2.1.2) and adult (3.1.2.1.3) of the mite species. The efficacies of the fractions were compared with those of neem oil emulsion (2 %) and horticultural mineral oil (2.5 %). An untreated control was also maintained. The experiment was laid out in Completely Randomized Design with six treatments, each replicated thrice with eight plants per replication (Table. 4). Treatments were imposed 15 days after the release of the mites, using a hand sprayer (Plate. 15).

Plate 13. Pot culture experiment in amaranthus

Plate 14. Release of mites on amaranthus plants

Plate 15. Spraying with solvent fractions

Plate 16. Counting mite population

For recording the mite population in treatments, three plants were selected randomly from each treatment replication. Number of mites (both egg and active stages) was recorded from three windows of 1 cm^2 area, each from three leaves per plant representing the top, middle and bottom canopy of the plant (Plate. 16). 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 (Laya, 2020).

The data on mean number of mites per cm^2 leaf area at 1, 3, 7, 10 and 14 days after treatment were subjected to analysis of variance (ANOVA) using the software, GRAPES 1.0.0, developed by Kerala Agricultural University. The result obtained was subjected to LSD (Least Significance Difference Test). The mean per cent reduction in population was also worked out at 7 and 14 days after treatment application.

Table.4. Experimental design for evaluation of selected solvent fractions against *Tetranychus truncatus* in amaranthus

Design : Completely Randomized Design (CRD)	
Crop : Amaranthus	Variety: Arun
No. of treatments : 6	No. of replications : 3
No. of plants/replications : 8	
T_1 : Hexane fraction (0.15 %)	T_4 : Neem oil emulsion (2 %)
T_2 : Chloroform fraction (0.2 %)	T_5 : Horticultural Mineral Oil (2.5 %)
T_3 : Aqueous fraction (0.2 %)	T_6 : Untreated control

3.3. Evaluation of selected botanical fractions against *Aphis craccivora* in pot culture

A pot culture experiment was conducted to evaluate the efficacy of the effective concentrations of the fractions of *T. minuta* against *Aphis craccivora* in cowpea. The experiment was laid out in the open area adjacent to the Insectary building, Department of Agricultural Entomology, during July - September, 2022 (Plate.17). One concentration each of hexane (0.2 %), chloroform (0.2 %) and aqueous (0.2 %) fractions

were chosen for evaluation, based on their efficacy in the laboratory study against the adults of the aphid (3.1.2.2.2). The efficacies of the fractions was compared with those of neem oil emulsion (2%), azadirachtin (1 % EC) (3ml/L) and horticultural mineral oil (2 %). An untreated control was also maintained. The experiment was laid out in Completely Randomized Design with seven treatments, each replicated thrice with eight plants per replication (Table. 5).

The bush type cowpea variety, Bhagyalakshmi was used for the study. The seeds were collected from the Department of Vegetable Science and sown in individual polybags of size 35 cm x 20 cm x 20 cm, filled with potting mixture (soil, coir pith compost and cow dung in 2:1:1 ratio). All cultural practices were carried out as per Package of Practices Recommendations, KAU (2016). Aphids were allowed to infest the 20 days old cowpea plants by keeping aphid infested cowpea sprouts in paper cups at the base of each plant in polybag. Treatments were imposed 20 days after the release of aphids using a hand sprayer (Plate. 18). For recording the aphid population in treatments, three plants were selected randomly from each treatment replication. Number of aphids (both nymphs and adults) was recorded from three randomly collected shoot bits of 5 cm length, excised from tender parts of each plant (Plate. 19) (Bindu, 1997). Pre-treatment count one day prior to treatment and post treatment counts of aphids at 1, 3, 7 and 10 days after treatment application were recorded by dislodging the aphids on each shoot bit on to a white paper.

The data on mean number of aphids per 5 cm shoot length before and after treatments were subjected to analysis of covariance (ANCOVA) using the software, GRAPES 1.0.0, developed by Kerala Agricultural University. In order to accommodate the variations in pre-count, the transformed data were analysed by taking population counts prior to the first application as covariate and ANCOVA was done for observations at 1,3, 7 and 10 days after treatment. The result obtained was subjected to LSD (Least Significance Difference Test). The mean per cent reduction in population was also worked out ten days after treatment application.

Plate 17. Pot culture experiment in cowpea

Plate 18. Spraying with solvent fractions

Plate 19a. Selection of shoot bits (5 cm length)

Plate 19b. Excising shoot bits

Plate 19. Counting aphid population

Table 5. Experimental design for evaluation of selected solvent fractions against *Aphis craccivora* in cowpea

Design : Completely Randomized Design (CRD)	
Crop : Cowpea Variety: Bhagyalakshmi	
No. of treatments : 7 No. of replications : 3	
No. of plants/replications : 8	
T_1 : Hexane fraction (0.2 %)	T_5 : Azadirachtin (1 % EC) (3ml/L)
T_2 : Chloroform fraction (0.2 %)	T_6 : Horticultural Mineral Oil (2 %)
T_3 : Aqueous fraction (0.2 %)	T_7 : Untreated control
T_4 : Neem oil emulsion (2 %)	

Results

4. RESULTS

Results of the study on 'Bio-efficacy of *Tagetes minuta* L. against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae) and *Aphis craccivora* Koch (Hemiptera: Aphididae), based on the experiments conducted in the laboratory and fields of All India Network Project on Agricultural Acarology, Department of Agricultural Entomology, are presented in this chapter.

4.1. Identification of bioactive solvent fraction of *Tagetes minuta* against *Tetranychus truncatus* and *Aphis craccivora*

4.1.1. Extraction of solvent fractions of *Tagetes minuta*

The botanical *T. minuta* was extracted sequentially using three different solvents *viz*., hexane, chloroform and water. The solvent fractions of hexane, chloroform and water recorded an yield of 3.11, 2.90 and 4.72 per cent, respectively (Table 6).

Table 6. Yield of solvent fractions of *Tagetes minuta*

Sl. No.	*T. minuta* solvent fraction	Yield (%)
1	Hexane fraction	3.11
2	Chloroform fraction	2.90
3	Aqueous fraction	4.72

4.1.2. Laboratory evaluation of bioefficacy of *Tagetes minuta* solvent fractions

Laboratory study was carried out to evaluate the bioefficacy of the solvent fractions of *T. minuta* against against *T. truncatus* and *A. craccivora.*

4.1.2.1. Bioefficacy of *Tagetus minuta* fractions against *Tetranychus truncatus*

The efficacy of the solvent fractions against the eggs and adults of *T. truncatus* was evaluated separately.

4.1.2.1.1. Ovicidal effect of solvent fractions

The hexane, chloroform and aqueous fractions of *T. minuta* were evaluated for ovicidal action against *T. truncatus* at five different concentrations *viz*., 0.025, 0.05, 0.1, 0.15 and 0.2 %. The botanical fractions varied in their ovicidal action at different concentrations and time intervals (Table. 7).

The eggs did not hatch until 48 h, in any of the treatments tested. At third day (72h) of treatment, no hatching was observed in hexane fraction (0.2 %). Among other treatments, significantly lower hatchability was observed in hexane fraction (0.15 %), followed by hexane fraction (0.1 %) and aqueous fraction (0.2 %) with 9.33, 20.00 and 37.33 per cent hatchability, respectively. The aqueous fraction (0.15 %), chloroform fraction (0.2 %) and hexane fraction (0.05 %) showed per cent hatchability of 42.67, 44.00 and 45.33, respectively, and were on par with one another. Chloroform fraction (0.15 %) and aqueous fraction (0.1 %) were on par with each other showing per cent hatchability of 52.00 and 53.33, respectively. Significantly higher per cent hatchability of 54.67, 58.67, 60.00, 62.67, 66.70 and 70.67 were exhibited by chloroform fraction (0.1 %), hexane fraction (0.025 %), chloroform fraction (0.05 %), aqueous fraction (0.05 %), aqueous fraction (0.025 %) and chloroform fraction (0.025 %), respectively. Hatchability in control treatments were 72.00 and 73.33 per cent.

After 96 h of treatment, hexane fraction (0.2 %) continued to show zero hatchability, while hexane fraction (0.15 %) exhibited a significantly lower hatchability of 14.67 per cent, compared to other treatments. This was followed by hexane fraction (0.1 %) that showed 34.67 per cent hatchability. Aqueous fraction (0.2 %) and hexane fraction (0.05 %) were on par with each other, recording per cent hatchability of 62.67 and 69.33, respectively. The chloroform fraction (0.2 %) and aqueous fraction (0.15 %) were on par with each other showing per cent hatchability of 74.67 and 76.00, respectively. Similarly, chloroform fraction (0.15 %) and aqueous fraction (0.1 %) were on par, recording per cent hatchability of 82.67 and 82.70. Aqueous fraction (0.05 %) exhibited 84.00 per cent hatchability, while hexane fraction (0.025 %) and chloroform fraction (0.1 %) were on par, both showing a per cent hatchability of 85.33. Other treatments, *viz*., aqueous fraction (0.025 %), chloroform fraction (0.05 %) and

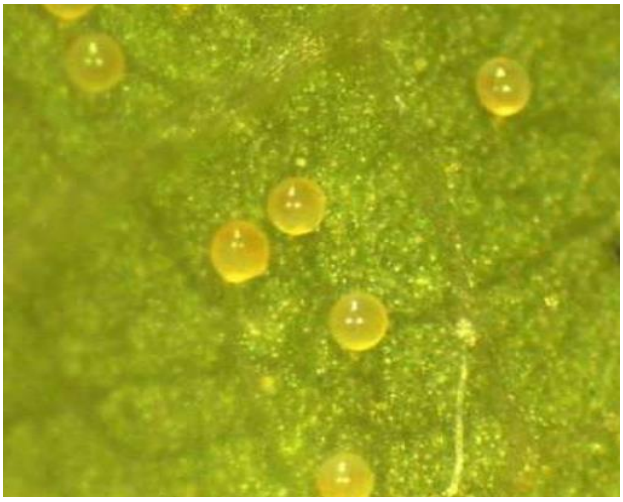

Plate 20a. Live eggs

Plate 20b. Unhatched eggs after 5 days

Plate 20. Effect of 0.2 % hexane fraction of *Tagetes minuta* on eggs of *Tetranychus truncatus*

chloroform fraction (0.025 %) recorded significantly higher hatchability of 92.00, 93.33 and 98.70 per cent, respectively. Hatchability in control treatments were 98.67 and 97.33 per cent.

Even after the fifth day (120 h) of treatment, all eggs treated with hexane fraction (0.2 %) failed to hatch. The hexane fraction (0.15 %) showed the next lower hatchability of 18.67 per cent, followed by hexane fraction (0.1 %) with 46.67 per cent hatchability. Other treatments, namely: chloroform fraction (0.2 %), aqueous fraction (0.2 %), chloroform fraction (0.15 %), hexane fraction (0.05 %), aqueous fraction (0.15 %), aqueous fraction (0.05 %), chloroform fraction (0.1 %), aqueous fraction (0.1 %), aqueous fraction (0.025 %) and hexane fraction (0.025 %) recorded significantly higher level of hatchability, *viz.*, 80.00, 81.33, 86.67, 88.00, 89.33, 93.33, 93.33, 94.67, 97.33 and 98.67 per cent, respectively. However, cent per cent hatchability was observed in the case of chloroform fractions at 0.025 and 0.05 per cent, which were on par with the control treatments.

Per cent mortality of eggs was calculated after 120 h of treatment. Mortality of 100 per cent was observed for hexane fraction (0.2 %), which was found superior to all other treatments. This was followed hexane fraction at by 0.15 and 0.1 per cent, which caused 81.33 and 53.33 per cent mortality of eggs, respectively. Mortality of eggs was significantly lower for all other treatments. Chloroform fraction (0.2 %), aqueous fraction (0.2 %), chloroform fraction (0.15 %), hexane fraction (0.05 %), aqueous fraction (0.15 %), chloroform fraction (0.1 %), aqueous fraction (0.05 %), aqueous fraction (0.1 %), aqueous fraction (0.025 %) and hexane fraction (0.025 %) recorded per cent mortality of 20.00, 18.67, 13.30, 12.00, 10.67, 6.67, 6.67, 5.33, 2.67 and 1.33, respectively. However, no mortality was observed in chloroform fractions at 0.025 and 0.05 per cent.

Table 7. Ovicidal effect of *Tagetes minuta* solvent fractions on *Tetranychus truncatus*

Treatment	Hatchability (%)					Mortality at 120 h (%)
	24 h	48 h	72 h	96 h	120 h	
Hexane fraction (0.025 %)	0.00 (0.57)	0.00 (0.57)	58.67^{cde} (50.01)	85.33^{cde} (67.81)	98.67^{ab} (86.15)	1.33^{jk} (3.85)
Hexane fraction (0.05 %)	0.00 (0.57)	0.00 (0.57)	45.33^{fg} (42.31)	69.33^{f} (56.38)	88.00^{efgh} (69.71)	12.00^{defg} (20.09)
Hexane fraction (0.1 %)	0.00 (0.57)	0.00 (0.57)	20.00^{h} (26.49)	34.67^{g} (36.02)	46.67^{i} (43.08)	53.33^{c} (46.92)
Hexane fraction (0.15 %)	0.00 (0.57)	0.00 (0.57)	9.33^{i} (14.62)	14.67^{h} (22.19)	18.67^{j} (25.50)	81.33^{b} (64.50)
Hexane fraction (0.2 %)	0.00 (0.57)	0.00 (0.57)	0.00^{j} (0.57)	0.00^{i} (0.57)	0.00^{k} (0.57)	100.00^{a} (89.43)
Chloroform fraction (0.025 %)	0.00 (0.57)	0.00 (0.57)	70.67^{abc} (57.22)	98.70^{a} (86.15)	100.00^{a} (89.43)	0.00^{k} (0.57)
Chloroform fraction (0.05 %)	0.00 (0.57)	0.00 (0.57)	60.00^{bcde} (50.82)	93.33^{cd} (75.20)	100.00^{a} (89.43)	0.00^{k} (0.57)
Chloroform fraction (0.1 %)	0.00 (0.57)	0.00 (0.57)	54.67^{def} (47.68)	85.33^{cde} (67.81)	93.33^{cd} (77.77)	6.67^{hi} (12.23)
Chloroform fraction (0.15 %)	0.00 (0.57)	0.00 (0.57)	52.00^{ef} (46.15)	82.67^{e} (65.43)	86.67^{fgh} (68.63)	13.30^{def} (21.37)
Chloroform fraction (0.2 %)	0.00 (0.57)	0.00 (0.57)	44.00^{fg} (41.55)	74.67^{ef} (59.79)	80.00^{h} (63.51)	20.00^{d} (26.49)
Aqueous fraction (0.025 %)	0.00 (0.57)	0.00 (0.57)	66.70^{abcd} (54.85)	92.00^{bc} (76.49)	97.33^{bc} (82.31)	2.67^{ij} (7.69)
Aqueous fraction (0.05 %)	0.00 (0.57)	0.00 (0.57)	62.67^{abcde} (52.38)	84.00^{de} (66.53)	93.33^{cdef} (75.20)	6.67^{fghi} (14.80)

Aqueous fraction (0.1 %)	0.00 (0.57)	0.00 (0.57)	53.33^{ef} (46.92)	82.70^{e} (65.53)	94.67^{cde} (76.83)	5.33^{ghi} (13.17)
Aqueous fraction (0.15 %)	0.00 (0.57)	0.00 (0.57)	42.67^{fg} (40.72)	76.00^{ef} (60.72)	89.33^{defg} (71.54)	10.67^{efgh} (18.46)
Aqueous fraction (0.2 %)	0.00 (0.57)	0.00 (0.57)	37.33^{g} (37.62)	62.67^{f} (52.38)	81.33^{gh} (64.61)	18.67^{de} (25.39)
Control (Water + emulsifier)	0.00 (0.57)	0.00 (0.57)	73.33^{a} (59.08)	98.67^{a} (86.15)	100.00^{a} (90.00)	0.00^{k} (0.57)
Absolute control	0.00 (0.57)	0.00 (0.57)	72.00^{ab} (58.09)	97.33^{ab} (84.52)	100.00^{a} (90.00)	0.00^{k} (0.57)
LSD (0.05)	**-**	**-**	**7.662**	**8.721**	**7.414**	**7.414**

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%

4.1.2.1.2. Adulticidal effect of solvent fractions

The solvent fractions of *T. minuta* at five different concentrations *viz.*, 0.025, 0.05, 0.1, 0.15 and 0.2 per cent were evaluated for adulticidal action against *T. truncatus*. Since, there was natural mortality in control, the mortalities were corrected by Abbott's formula. The results showed that the treatments varied significantly in their efficacy against the adult mites (Table.8).

After 24 h of treatment, hexane fraction at 0.2, 0.15 and 0.1 per cent recorded significantly higher mortality on par with one another, causing 27.40 , 26.03 and 24.66 per cent mortality of the adults. This was followed by chloroform fraction (0.2 %) recording 17.81 per cent mortality. Aqueous fractions at 0.2 and 0.15 per cent recorded adult mortality of 16.44 and 15.07 per cent, respectively. Chloroform fraction (0.1 %), aqueous fraction (0.1 %) and hexane fraction (0.05 %) recorded significant adult mortality of 13.70, 10.14 and 9.59 per cent, respectively. Aqueous fraction at 0.05 and 0.025 per cent and chloroform fraction at 0.025 per cent were on par with one another,

all recording 8.22 per cent mortality in adult mites. Mortality in other treatments, *viz.*, 0.15 and 0.05 per cent chloroform fraction and 0.025 per cent hexane fraction were 6.85, 4.11 and 2.74 per cent and were significantly inferior to the above treatments.

After 48 h of treatment, hexane fraction at 0.2, 0.15, 0.1 and 0.05 per cent recorded significantly higher per cent mortality of 52.17, 57.97, 46.38 and 46.38, respectively and were on par with one another. This was followed by chloroform fraction (0.2 %) that caused a mortality of 31.88 per cent in adult mites. The hexane fraction (0.025 %) chloroform fraction (0.1 %) and aqueous fraction (0.2 %) were found to be on par with one another, recording 28.99 per cent mortality. Aqueous and chloroform fractions at 0.15 per cent exhibited adult mortality of 21.74 and 17.39 per cent, followed by aqueous fraction (0.1 %) (10.96 % mortality). Other four treatments, *viz*. aqueous fraction at 0.05 and 0.025 per cent and chloroform fraction at 0.05 and 0.025 per cent were on par with one another, recording significantly lower per cent mortality of 8.70, 8.70, 7.25 and 8.70, respectively compared to the above treatments.

At 72 h of treatment, both 0.2 and 0.15 per cent hexane fractions recorded significantly higher mortality of 74.24 per cent. Hexane fraction (0.1 %), hexane fraction (0.05 %), chloroform fraction (0.2 %) and chloroform fraction (0.15 %) also caused significant level of mortality of 53.03, 48.48, 43.94 and 39.39 per cent, respectively. This was followed by chloroform fraction (0.1 %) and hexane fraction (0.025 %) that recorded 37.88 and 34.85 per cent mortality in adult mites. Aqueous fractions at 0.2 and 0.15 per cent recorded mortality of 31.82 and 27.27 per cent, respectively. All other treatments recorded significantly lower mortality. Of these treatments, chloroform fraction (0.025 %) and aqueous fraction (0.1 %) were on par with each other, showing 13.64 per cent mortality, followed by aqueous fraction (0.05 %) recording a mortality of 12.12 per cent. Similarly, aqueous fraction (0.025 %) and chloroform fraction (0.05 %) were on par, recording 9.09 per cent mortality.

After 96 h of treatment, 0.2 and 0.15 per cent hexane fractions continued to be superior to other treatments recording per cent adult mortality of 79.69 and 85.94, respectively and were on par with each other. These treatments were followed by hexane fraction (0.1 %) that recorded 60.94 per cent mortality. Chloroform fraction

Plate 21a. Live adults

Plate 21a. Dead adults

Plate 21. Effect of 0.2 % hexane fraction of *Tagetes minuta* on adults of *Tetranychus truncatus*

(0.2%) and hexane fraction (0.05 %) were on par with each other, causing 59.38 and 57.81 per cent mortality, respectively, in adult mites. Chloroform fractions at both 0.15 and 0.1 per cent recorded 48.44 per cent mortality. Aqueous fraction (0.2 %) and hexane fraction (0.025 %) also recorded significant mortality of 45.31 and 42.19 per cent, respectively. In other treatments, mortality was lower than the above treatments, though significantly higher than control. The treatments recorded mortality of 31.25, 28.13, 21.88, 21.88, 18.75 and 14.06 per cent, respectively in aqueous fraction (0.15 %), chloroform fraction (0.025 %), chloroform fraction (0.05 %), aqueous fraction (0.1 %), aqueous fraction (0.05 %) and aqueous fraction (0.025 %).

By 120 h of treatment, 0.2 and 0.15 per cent hexane fractions recorded significantly higher mortality of 91.67 and 90.00 per cent, respectively and were on par with each other. This was followed by chloroform fraction (0.2 %) and hexane fraction (0.1 %), recording 66.67 and 65.00 per cent adult mortality, respectively. Chloroform fraction at both 0.1and 0.15 per cent showed significant mortality of 56.67 per cent. Mortality in aqueous fraction (0.2 %) and hexane fraction (0.025 %) were 53.33 and 51.67 per cent, respectively. The 0.15 and 0.1 per cent aqueous fractions were on par with each other with lower per cent mortality of 38.33 and 33.33, respectively. Mortality was much lower in 0.05 and 0.025 per cent chloroform fractions, recording 30.00 and 28.33 per cent adult mortality, respectively. Treatments involving 0.05 and 0.025 per cent aqueous fractions were significantly inferior to the above treatments, recording lower adult mortality of 20.00 and 16.67 per cent, respectively.

The results showed that all the three fractions, at the different concentrations tested, were having significantly high efficacy against adult mites. However, the hexane fraction at 0.2 and 0.15 per cent were significantly much superior to all other treatments. Aqueous fraction showed the least effect against adults of *T. truncatus.*

Table 8. Adulticidal effect of *Tagetes minuta* solvent fractions on *Tetranychus truncatus*

Treatment	Mortality (%)				
	24 h	48 h	72 h	96 h	120 h
Hexane fraction (0.025 %)	2.74 ^{hi} (9.53)	28.99 ^{bc} (32.57)	34.85 ^{cde} (36.18)	42.19 ^{de} (40.51)	51.67 ^{d} (45.96)
Hexane fraction (0.05 %)	9.59 ^{defg} (18.04)	46.38 ^{a} (42.92)	48.48^{bc} (44.13)	57.81 ^{bc} (49.49)	61.67 ^{bcd} (51.75)
Hexane fraction (0.1 %)	24.66^{a} (29.77)	46.38 ^{a} (42.92)	53.03 ^{b} (46.74)	60.94 ^{b} (51.32)	65.00 ^{bc} (53.73)
Hexane fraction (0.15 %)	26.03 ^{a} (30.68)	57.97 ^{a} (49.59)	74.24 ^{a} (59.50)	85.94 ^{a} (67.98)	90.00 ^{a} (71.57)
Hexane fraction (0.2 %)	27.40 ^{a} (31.56)	52.17 ^{a} (46.25)	74.24 ^{a} (59.50)	79.69 ^{a} (63.21)	91.67 ^{a} (73.22)
Chloroform fraction (0.025 %)	8.22 ^{efgh} (16.66)	8.70^{ef} (17.15)	13.64 ^{fg} (21.67)	28.13 ^{fg} (32.03)	28.33 ^{efg} (32.16)
Chloroform fraction (0.05 %)	4.11 ^{ghi} (11.70)	7.25 ^{ef} (15.62)	9.09^{gh} (17.55)	21.88 ^{fgh} (27.89)	30.00 ^{ef} (33.21)
Chloroform fraction (0.1 %)	13.70 ^{bcde} (21.72)	28.99 ^{bc} (32.57)	37.88 ^{cde} (37.99)	48.44 ^{cd} (44.10)	56.67 ^{bcd} (48.83)
Chloroform fraction (0.15 %)	6.85 ^{fgh} (15.17)	17.39 ^{cde} (24.65)	39.39 ^{bcde} (38.88)	48.44 ^{cd} (44.10)	56.67 ^{bcd} (48.83)
Chloroform fraction (0.2 %)	17.81 ^{b} (24.96)	31.88 ^{b} (34.38)	43.94 ^{bcd} (41.52)	59.38 ^{bc} (50.40)	66.67 ^{b} (54.74)
Aqueous fraction (0.025 %)	8.22 ^{efgh} (16.66)	8.70 ^{ef} (17.15)	9.09 ^{gh} (17.55)	14.06 ^{h} (22.02)	16.67 ^{g} (24.09)
Aqueous fraction (0.05 %)	8.22 ^{efgh} (16.66)	8.70 ^{ef} (17.15)	12.12 ^{g} (20.37)	18.75 ^{gh} (25.66)	20.00 ^{fg} (26.57)
Aqueous fraction (0.1 %)	10.14 ^{cdef} (19.33)	10.96 ^{def} (18.57)	13.64 ^{fg} (21.67)	21.88 ^{fgh} (27.89)	33.33 ^{e} (35.26)

Aqueous fraction (0.15 %)	15.07 ^{bcd} (22.84)	21.74 ^{bcd} (27.79)	27.27 ^{ef} (31.48)	31.25 ^{ef} (33.99)	38.33 ^{e} (38.25)
Aqueous fraction (0.2 %)	16.44 ^{bc} (23.92)	28.99 ^{bc} (32.57)	31.82 ^{de} (34.34)	45.31 ^{d} (42.31)	53.33 ^{cd} (46.91)
LSD (0.05)	**6.119**	**8.176**	**7.813**	**6.435**	**6.017**

Data represents corrected mortality after Abbott's correction
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. Bioefficacy of *Tagetus minuta* solvent fractions against *Aphis craccivora*

The hexane, chloroform and aqueous fractions of *T. minuta*, at five different concentrations (0.025, 0.05, 0.1, 0.15 and 0.2 %) were tested for their efficacy against adults of *A. craccivora*. Data on the reduction in adult count caused by the treatments (due to repellant action) at different time intervals is presented in Table 9. The results showed that the treatments varied significantly in repelling the adult aphids.

After 24 h of treatment, hexane fraction (0.2 %) recorded maximum reduction in aphid numbers by repelling 61.67 per cent of the adult aphids and it was found to be significantly superior to all other treatments. This was followed by 0.15 per cent hexane fraction and 0.2 per cent chloroform fraction (60.00 %), and 0.2 per cent aqueous fraction (58.33 %) which were on par with one another. Aqueous fraction (0.15 %) reduced adult aphid numbers by 50.00 per cent, followed by 0.15 per cent chloroform fraction (48.33 %), 0.1 per cent hexane fraction (43.33%) and 0.05 per cent hexane fraction (40.00 %). Chloroform fraction (0.1 %) was on par with aqueous fraction (0.1 %), each recording 38.33 per cent reduction in aphid population. Chloroform fraction at 0.05 and 0.025 per cent reduced aphid numbers by 36.67 and 30.00 per cent, respectively. In other treatments, namely hexane fraction (0.025 %) and aqueous fraction at 0.05 and 0.025 per cent, reduction in aphid numbers were 23.33, 28.33 and 15.00 per cent, respectively. In the control treatments, 13.33 and 1.67 per cent reduction were recorded.

At 48 h of treatment, hexane fraction (0.2 %) that recorded a population reduction of 83.33 per cent was found to be significantly superior to all other treatments, followed by chloroform fraction (0.2 %), hexane fraction (0.15 %), chloroform fraction (0.15 %) and aqueous fraction (0.2 %) that reduced adult aphid numbers by 78.33, 75.00, 73.33 and 71.67 per cent, respectively. Chloroform fraction at 0.1 and 0.05 per cent recorded 66.67 and 65 per cent reduction in adult aphids. Hexane fraction (0.1 %) and aqueous fraction (0.15 %) were at par, showing 63.33 per cent reduction in aphid count. This was followed by aqueous fraction (0.1 %), hexane fraction (0.05 %), chloroform fraction (0.025 %), aqueous fraction (0.05 %), hexane fraction (0.025 %) and aqueous fraction (0.025 %) that recorded a reduction of 61.67, 53.33, 48.33, 45.00, 40.00 and 36.67 per cent, respectively. The control treatments recorded 13.33 and 10.00 per cent reduction in aphid numbers.

After 72 h of treatment, hexane and chloroform fractions at 0.2 per cent were at par, recording 86.67 and 85.00 per cent reduction in aphid count. Aqueous fraction (0.2 %), chloroform fraction (0.15 %) and hexane fraction (0.15 %) also recorded significantly high reduction of 81.67, 80.00 and 76.67 per cent, respectively. Aqueous fraction (0.15 %) and chloroform fraction (0.1 %) were at par, reducing aphid numbers by 71.67 per cent. This was followed by chloroform fraction (0.05 %) and hexane fraction (0.1 %), aqueous fraction (0.1 %), hexane fraction (0.05 %) and chloroform fraction (0.025 %) which reduced adult count by 70.00, 68.33, 65.00, 60.00 and 55.00 per cent, respectively. Aqueous fraction (0.05 %) and 0.025 % hexane fraction were at par, each recording 53.33 per cent reduction followed by aqueous fraction (0.025 %) (45.00 % reduction). In control treatments, the count of adult aphids was reduced by only 16.67 and 11.67 per cent

At 96 h of treatment, hexane fraction (0.2 %) was superior to all other treatments, recording 88.33 per cent reduction in aphid count. This was followed by chloroform fraction (0.2 %), aqueous fraction (0.2 %), chloroform fraction (0.15 %) and hexane fraction (0.15 %) which reduced the aphid count significantly by 86.67 , 83.33 , 81.67 and 80.00 per cent, respectively. Hexane fraction (0.1 %), chloroform fraction (0.1 %) and aqueous fraction (0.15 %) were on par with one another, all recording significant reduction of 75.00 per cent in aphid numbers. Similarly, aqueous

fraction (0.1 %) and chloroform fraction (0.05 %), that recorded 70.00 and 71.67 per cent reduction in aphid count, respectively, were on par with each other. The hexane fraction (0.05 %) reduced the aphid number by 65.00 per cent, followed by hexane and chloroform fractions at 0.025 per cent, each causing 56.67 per cent reduction. Aqueous fraction at 0.05 and 0.025 per cent reduced aphid numbers by 55.00 and 46.67 per cent, respectively, while the control treatments reduced aphid numbers by 18.33 and 13.33 per cent only.

After 120 h of treatment, hexane fraction (0.2 %), hexane fraction (0.15 %), chloroform fraction (0.2 %), chloroform fraction (0.15 %) and aqueous fraction (0.2 %) recorded reduction in aphid count of 90.00, 86.67, 86.67, 85.00 and 85.00 per cent and were on par with one another. These treatments were significantly superior to all other treatments. This was followed by 0.15 % aqueous fraction that reduced aphid numbers by 78.33 per cent. Chloroform fraction (0.1 %), hexane fraction (0.1 %) and chloroform fraction (0.05 %) were on par with one another with 76.67, 76.67 and 73.33 per cent reduction, followed by aqueous fraction (0.1 %) and hexane fraction (0.05 %) that recorded 70.00 and 65.00 per cent reduction, respectively. Aqueous fraction (0.05 %) and chloroform fraction (0.025 %) were on par with each other, exhibiting 58.33 per cent reduction. Hexane fraction (0.025 %) reduced aphid count by 56.67 per cent, followed by aqueous fraction (0.025 %) which caused the least population reduction among all the above treatments (48.33 %). However, all treatments were significantly superior to the control treatments, which recorded only 18.33 and 13.33 per cent reduction in of adult aphids.

Table 9. Bioefficacy of *Tagetes minuta* solvent fractions against *Aphis craccivora*

Treatment	Reduction in aphid count (%)				
	24 h	**48 h**	**72 h**	**96 h**	**120 h**
Hexane fraction (0.025 %)	23.33 ^{fg} (28.67)	40.00 ^{hi} (39.21)	53.33 ^{gh} (46.92)	56.67 ^{gh} (48.84)	56.67 ^{f} (48.84)
Hexane fraction (0.05 %)	40.00 ^{cde} (39.21)	53.33 ^{fg} (46.94)	60.00 ^{fg} (50.85)	65.00 ^{fg} (53.76)	65.00 ^{de} (53.76)
Hexane fraction (0.1 %)	43.33 ^{cd} (41.13)	63.33 ^{e} (52.78)	68.33^{def} (55.82)	75.00 ^{de} (60.07)	76.67^{bc} (61.15)
Hexane fraction (0.15 %)	60.00 ^{ab} (50.79)	75.00 ^{b} (60.07)	76.67 ^{bcd} (61.15)	80.00^{cd} (63.55)	86.67 ^{a} (68.66)
Hexane fraction (0.2 %)	61.67 ^{a} (51.81)	83.33 ^{a} (65.95)	86.67 ^{a} (68.66)	88.33 ^{a} (70.11)	90.00 ^{a} (71.57)
Chloroform fraction (0.025 %)	30.00 ^{ef} (33.16)	48.33 ^{gh} (44.04)	55.00 ^{gh} (47.88)	56.67 ^{gh} (48.84)	58.33^{ef} (49.80)
Chloroform fraction (0.05 %)	36.67 ^{de} (37.20)	65.00 ^{de} (53.76)	70.00 ^{de} (56.84)	71.67 ^{ef} (57.86)	73.33^{bc} (59.00)
Chloroform fraction (0.1 %)	38.33 ^{cde} (38.19)	66.67 ^{cde} (54.75)	71.67 ^{cde} (57.86)	75.00 ^{de} (60.00)	76.67 ^{bc} (61.15)
Chloroform fraction (0.15 %)	48.33^{bcd} (44.01)	73.33 ^{bc} (58.93)	80.00 ^{abc} (63.55)	81.67 ^{bcd} (64.81)	85.00 ^{a} (67.40)
Chloroform fraction (0.2 %)	60.00 ^{ab} (50.79)	78.33 ^{ab} (62.29)	85.00 ^{a} (67.40)	86.67 ^{ab} (68.66)	86.67 ^{a} (68.66)
Aqueous fraction (0.025 %)	15.00 ^{gh} (22.29)	36.67 ^{i} (37.26)	45.00 ^{h} (42.12)	46.67 ^{i} (43.09)	48.33 ^{g} (44.04)
Aqueous fraction (0.05 %)	28.33 ^{ef} (32.14)	45.00 ^{ghi} (42.12)	53.33 ^{gh} (46.92)	55.00^{hi} (47.91)	58.33 ^{ef} (49.83)
Aqueous fraction (0.1 %)	38.33 ^{cde} (38.22)	61.67 ^{ef} (51.76)	65.00 ^{ef} (53.76)	70.00 ^{ef} (56.79)	70.00 ^{cd} (56.79)
Aqueous fraction (0.15 %)	50.00 ^{abc}	63.33 ^{e}	71.67 ^{cde}	75.00 ^{de}	78.33^{b}

	(45.00)	(52.74)	(57.86)	(60.07)	(62.29)
Aqueous fraction (0.2 %)	58.33 ^{ab} (49.80)	71.67 ^{bcd} (57.86)	81.67 ^{ab} (65.00)	83.33 ^{abc} (66.15)	85.00 ^{a} (67.40)
Control (Water + emulsifier)	13.33 ^{h} (21.34)	13.33 ^{j} (21.34)	16.67 ^{i} (24.05)	18.33 ^{j} (25.31)	18.33^{h} (25.31)
Absolute control	1.67^{i} (4.31)	10.00 ^{j} (18.43)	11.67 ^{i} (19.89)	13.33 ^{j} (21.34)	13.33 ^{h} (21.34)
LSD (0.05)	**7.329**	**4.881**	**5.789**	**4.974**	**4.568**

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 %

4.1.3. Qualitative phytochemical analysis

Qualitative phytochemical analysis of the hexane and chloroform solvent fractions by GC-MS/MS analysis yielded the following major bio-active compounds:

Table 10. Bio-active compounds in the hexane and chloroform fractions of *Tagetes minuta*

Solvent fraction	Name of the compound	Molecular formula	Bio-active property recorded	Reference
Hexane fraction	4α-Phorbol 12,13-didecanoate	$C_{40}H_{64}O_8$	TRPV4 agonist	Alexander *et al*., 2012
	4-o-Methylphorbol 12, 13- didecanoate	$C_{41}H_{66}O_8$	TRPV4 agonist	Alexander *et al*., 2012
	Milbemycin b	$C_{33}H_{46}ClNO_7$	GABA gated chloride channel agonist	Ozoe, 2012
Chloroform fraction	4-o-Methylphorbol 12, 13- didecanoate	$C_{41}H_{66}O_8$	TRPV4 agonist	Alexander *et al*., 2012
	Milbemycin b	$C_{33}H_{46}ClNO_7$	GABA gated chloride channel agonist	Ozoe, 2012

4.2. Evaluation of selected botanical fractions against *Tetranychus truncatus* in pot culture

The best concentration each of the three solvent fractions, identified in the laboratory bioassays were evaluated against *T. truncatus* on amaranthus in a pot culture experiment.

The treatments were hexane fraction (0.15 %), chloroform fraction (0.2 %) and aqueous fraction (0.2 %) of *T. minuta*, along with neem oil emulsion (2%) and Horticultural Mineral Oil (HMO 2.5%). The mean number of mites before application of treatments ranged from 27.19 to 31.47 per cm^2 of leaf area (Table. 11).

After one day of treatment, the lowest mean mite population density was observed on plants treated with HMO (2.5 %) (14.90 per cm^2). This was followed by chloroform fraction (0.2 %) (18.38 per cm^2) and hexane fraction (0.15 %) (19.59 per cm^2), which were on par with each other. Neem oil emulsion (2 %) (24.61 per cm^2) and aqueous fraction (0.2 %) (26.05 per cm^2) recorded mite population on par with each other. The control treatment recorded a mite density of 32.07 per cm^2 leaf area.

Three days after treatment, plants treated with HMO (2.5 %) recorded significantly lower mite density of 10.20 per cm^2 leaf area, followed by 0.15 per cent hexane fraction (15.80 per cm^2), 0.2 per cent chloroform fraction (17.15 per cm^2), and 2 per cent neem oil emulsion (17.76 per cm^2) which were on par with one another. Aqueous fraction (0.2 %) recorded a mite density of 21.25 per cm^2, while in control, the mite density was 29.69 per cm^2 leaf area.

By the seventh day of treatment, the treatment involving HMO (2.5 %) was found to be significantly superior to all other treatments, recording a lower mite density of 6.40 per cm^2. The three treatments *viz.*, hexane fraction (0.15 %), chloroform fraction (0.2 %) and neem oil emulsion (2 %) recorded significantly lower mite population density of 12.39, 13.81 and 14.01 per cm^2 leaf area, respectively, and were on par with one another. This was followed by aqueous fraction (0.2 %) that recorded 17.85 mites per cm^2 leaf area. Mite population density in control was 28.70 per cm^2 leaf area. After seven days of spraying, HMO (2.5 %) recorded the highest per cent reduction in mite population (79.57 %), followed by hexane fraction (0.15 %), neem oil emulsion (2 %),

chloroform fraction (0.2 %) and aqueous fraction (0.2 %) that reduced mite population by 58.49, 52.92, 49.21 and 43.28 per cent, respectively.

Ten days after treatment, mite population was reduced to 4.64 per cm^2 leaf area in plants treated with HMO (2.5 %), followed by 9.38 per cm^2 in the treatment involving hexane fraction (0.15 %). The treatments chloroform fraction (0.2 %), aqueous fraction (0.2 %) and neem oil emulsion (2 %) recorded mite populations of 11.16, 11.58 and 11.67 per cm^2, respectively, and were on par with one another. Mite population in control was 29.06 per cm^2 leaf area.

After 14 days of treatment, HMO recorded the lowest mite population of 0.61 per cm^2 and was significantly superior to all other treatments. This was followed by hexane fraction (0.15 %) that recorded mite population of 3.35 per cm^2 leaf area. Chloroform fraction (0.2 %), neem oil emulsion (2 %) and aqueous fraction (0.2 %) were on par with one another, recording mite density of 5.99, 7.01 and 7.25 per cm^2, respectively. All the treatments recorded significantly lower mite density in comparison to control (32.27 mites per cm^2 leaf area). After 14 days from treatment, HMO (2.5 %) exhibited the highest per cent reduction in mite population (98.05 %), followed by 0.15 per cent hexane fraction (88.78 %). Other treatments *viz*., chloroform fraction (0.2 %), aqueous fraction (0.2 %) and neem oil emulsion (2 %) also recorded significant reduction in mite population of 77.97, 76.96 and 76.44 per cent, respectively. However, gradual build up in population was observed in control.

Table 11. Effect of treatments on *Tetranychus truncatus* in amaranthus

Sl. No	Treatment	Pre-count	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	14 DAT	
1	Hexane fraction (0.15 %)	29.85 (5.46)	19.59 [c] (4.42)	15.80 [c] (3.97)	12.39 [c] (3.52)	58.49	9.38 [c] (3.06)	3.35 [c] (1.82)	88.78
2	Chloroform fraction (0.2 %)	27.19 (5.21)	18.38 [c] (4.29)	17.15 [c] (4.14)	13.81 [c] (3.71)	49.21	11.16 [b] (3.34)	5.99 [b] (2.44)	77.97
3	Aqueous fraction (0.2 %)	31.47 (5.61)	26.05 [b] (5.10)	21.25 [b] (4.60)	17.85 [b] (4.22)	43.28	11.58 [b] (3.40)	7.25 [b] (2.69)	76.96
4	Neem oil emulsion (2 %)	29.76 (5.45)	24.61 [b] 4.96)	17.76 [c] (4.21)	14.01 [c] (3.74)	52.92	11.67 [b] (3.41)	7.01 [b] (2.64)	76.44
5	Horticultural Mineral Oil (2.5 %)	31.23 (5.58)	14.90 [d] (3.86)	10.20 [d] (3.19)	6.40 [d] (2.52)	79.51	4.64 [d] (2.15)	0.61 [d] (0.76)	98.05
6	Untreated control	31.14 (5.57)	32.07 [a] (5.66)	29.69 [a] (5.45)	28.70 [a] (5.35)	7.84	29.06 [a] (5.39)	32.27 [a] (5.68)	-
	LSD (0.05)	**_**	**0.334**	**0.294**	**0.339**	**_**	**0.163**	**0.338**	**_**

DAT- Days after treatment
Figures in parentheses are square root transformed values
Means followed by common letter(s) do not significantly differ at P=0.05 %

4.3. Evaluation of selected botanical fractions against *Aphis craccivora* in pot culture

An experiment was conducted to evaluate the efficacy of botanical fractions *viz.*, hexane fraction (0.2 %), chloroform fraction (0.2 %) and aqueous fraction (0.2 %) of *T. minuta* along with neem oil emulsion (2 %), azadirachtin 1 EC (3 ml/L) and Horticultural Mineral Oil (2 %) against *A. craccivora* on cowpea plant in pot culture. The mean number of aphids before application of treatments ranged from 33.93 to 80.37 per 5 cm of shoot length (Table. 12).

After one day of treatment, lowest aphid population was recorded in plants treated with HMO (2 %) (8.45 per 5 cm of shoot) and was significantly superior to all other treatments. This was followed by neem oil emulsion (2 %) and hexane fraction (2%) with 11.89 and 20.37 aphids per 5 cm of shoot, respectively. The other three treatments *viz*., chloroform fraction (0.2 %), azadirachtin 1 EC (3 ml/L) and aqueous fraction (0.2 %) were on par with one another, recording aphid population of 27.33, 29.00 and 30.04 per 5 cm of shoot length. Aphid population in control was 50.41 per 5 cm of shoot length.

Three days after treatment, mean aphid population in plants treated with HMO (2 %) was reduced to 6.48 per 5 cm of shoot, followed by 2 per cent neem oil emulsion (9.96) and 0.2 per cent hexane fraction (15.29). These were followed by aqueous fraction (0.2 %), chloroform fraction (0.2 %) and azadirachtin 1 EC (3 ml/L) that recorded aphid population of 20.74, 23.33 and 25.85 per 5 cm of shoot, respectively. Population in control was 44.36 per 5 cm of shoot length.

By the seventh day of treatment, aphid population remained significantly lower in HMO (2 %) and neem oil emulsion (2 %) recording 6.38 and 7.93 aphids, respectively, per 5 cm shoot and were on par with each other. This was followed by hexane fraction (2 %) that recorded 12.59 aphids. Aqueous fraction (0.2 %), chloroform fraction (0.2 %) and azadirachtin 1 EC (3 ml/L) were on par with one another, recording 18.63, 19.69 and 24.63 aphids, respectively. Mean number of aphids in untreated plants were 51.22 per 5 cm of shoot.

After ten days of spraying, HMO (2 %) was found to be significantly superior over other treatments recording mean aphid population of 5.59 per 5 cm shoot, while neem oil emulsion (2 %) recorded mean aphid population of 7.59 per 5 cm shoot. Hexane fraction (0.2 %) recorded aphid population of 11.58 per 5 cm of shoot and was on par with neem oil. Other treatments *viz*., chloroform fraction (0.2 %), aqueous fraction (0.2 %), and azadirachtin 1 EC (3 ml/L) recorded aphid populations of 18.41, 23.11 and 24.74, respectively, and were significantly superior to the control, where 61.78 aphids were observed per 5 cm of shoot.

Ten days post treatment, per cent reduction of aphid population was found to be higher in the treatments involving neem oil emulsion (2 %) and HMO (2 %), where aphid population was reduced by 87.59 and 83.52 per cent, respectively. This was followed by 78.95 per cent reduction in hexane fraction (0.2 %). Per cent reduction in other treatments *viz*., chloroform fraction (0.2 %), aqueous fraction (0.2 %) and azadirachtin 1 EC (3 ml/L) were 72.43, 68.20 and 67.97, respectively. However, gradual build up of aphid population was noticed in untreated plants.

Table. 12. Effect of treatments on *Aphis craccivora* in cowpea

Sl. No	Treatment	Mean no of live aphids/5 cm shoot length					Reduction in aphid population 10 DAT (%)
		Pre-count	1 DAT	3 DAT	7 DAT	10 DAT	
1	Hexane fraction (0.2 %)	53.11 (7.28)	20.37 ^{c} (4.51)	15.29 ^{d} (3.91)	12.59 ^{c} (3.54)	11.18 ^{d} (3.33)	78.95
2	Chloroform fraction (0.2 %)	66.78 (8.17)	27.33 ^{b} (5.22)	23.33 ^{bc} (4.81)	19.69 ^{b} (4.39)	18.41 ^{c} (4.27)	72.43
3	Aqueous fraction (0.2 %)	72.67 (8.52)	30.04 ^{b} (5.48)	20.74 ^{c} (4.55)	18.63 ^{b} (4.31)	23.11 ^{bc} (4.81)	68.20
4	Neem oil emulsion (2 %)	61.18 (7.82)	11.89 ^{d} (3.45)	9.96 ^{e} (3.16)	7.93 ^{d} (2.81)	7.59 ^{de} (2.75)	87.59
5	Azadirachtin 1 EC (3 ml/L)	80.37 (8.96)	29.00 ^{b} (5.38)	25.85 ^{b} (5.08)	24.63 ^{b} (4.95)	25.74 ^{b} (5.07)	67.97
6	Horticultural mineral oil (2 %)	33.93 (5.82)	8.45 ^{e} (2.90)	6.48 ^{f} (2.55)	6.38 ^{d} (2.52)	5.59 ^{e} (2.36)	83.52
7	Untreated control	43.74 (6.61)	50.41 ^{a} (7.09)	44.36 ^{a} (6.66)	51.22 ^{a} (7.15)	61.78 ^{a} (7.85)	–
	LSD (0.05)	**-**	**0.413**	**0.478**	**0.715**	**0.590**	**-**

DAT- Days after treatment
Figures in parentheses are square root transformed values
Means followed by common letter(s) do not significantly differ at P=0.05 %

Discussion

5. DISCUSSION

The findings of the study on efficacy of *Tagetes minuta* solvent fractions against the red spider mite, *Tetranychus truncatus* and the black cowpea aphid, *Aphis craccivora,* are discussed in this chapter in the light of available literature.

5.1. Identification of bioactive solvent fractions of *Tagetes minuta* against *Tetranychus truncatus* and *Aphis craccivora*

In the present study, efficacies of hexane, chloroform and aqueous fractions of the botanical, *Tagetes minuta* were evaluated against both eggs and adults of the mite, *T. truncatus* and adults of the aphid, *A. craccivora* in laboratory bioassays.

5.1.1. Bioefficacy of *Tagetes minuta* fractions against *Tetranychus truncatus*

In this study, the ovicidal and adulticidal effects of the different botanical fractions against *T. truncatus* varied considerably at various concentrations. Though very few studies have been carried out to evaluate the efficacy of the botanical, *T. minuta* against spider mites, several other botanicals were evaluated world-wide for their acaricidal properties against spider mites.

In the present study, significant ovicidal activity was exhibited only by the hexane fraction of the botanical, recording more than 50 per cent mortality at 0.1, 0.15 and 0.2 per cent concentrations. The lowest concentration tested (0.025 %) showed very less mortality of 1.33 per cent as compared to the highest concentration (0.2 %) which caused cent per cent mortality of *T. truncatus* eggs. The chloroform and aqueous fractions of *T. minuta* did not result in appreciable ovicidal effect of *T. truncatus,* with a maximum of only 20 per cent mortality in chloroform fraction (0.2 %) (Fig. 2). On the contrary, all the fractions (hexane, chloroform and aqueous) caused substantial

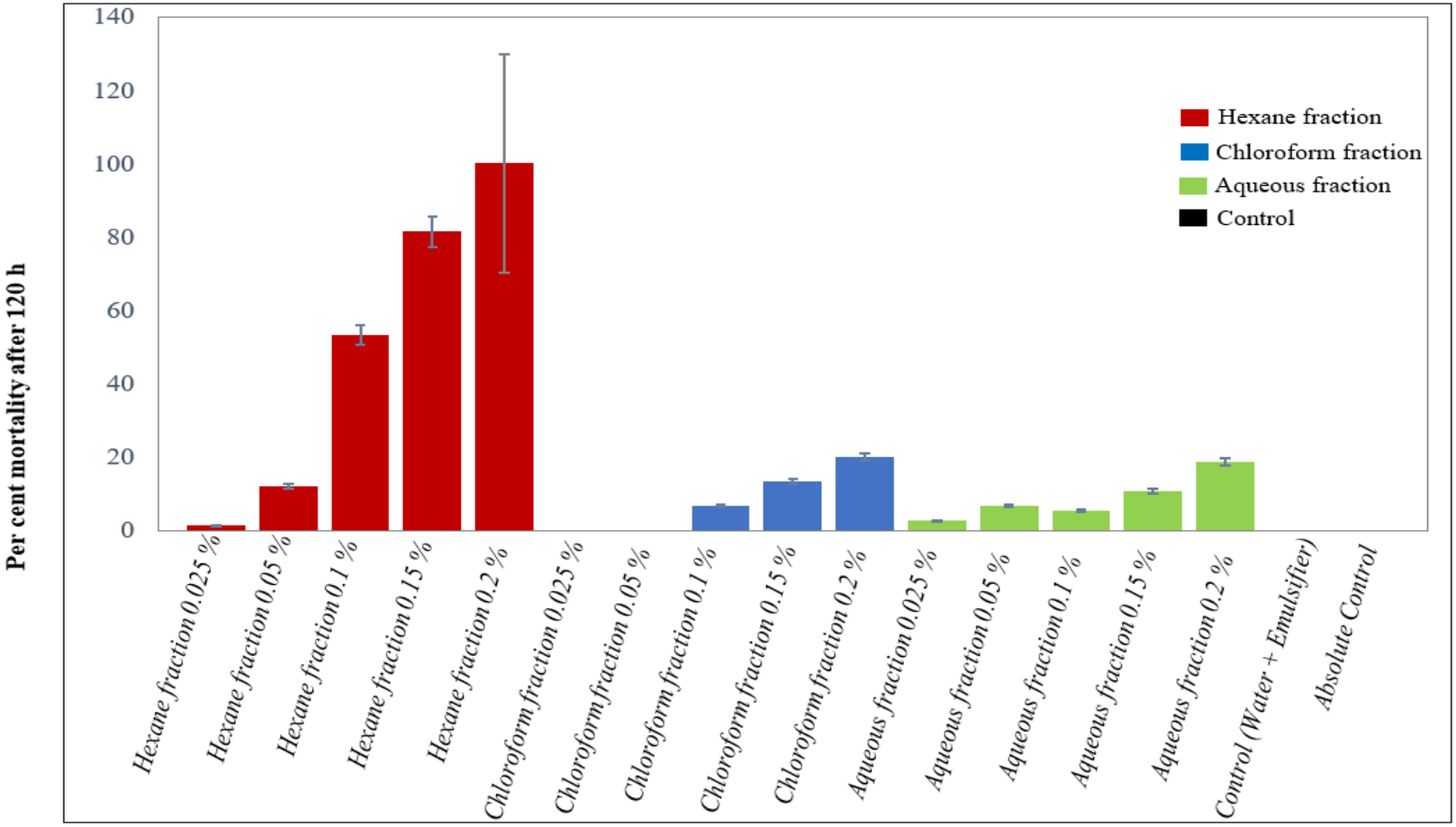

Fig. 2. Ovicidal effect of solvent fractions of *Tagetes minuta* on *Tetranychus truncatus*

mortality in adult mites. Even those concentrations which caused zero or negligible mortality of eggs, were able to cause considerable adult mortality. A typical example is the chloroform fraction at 0.025 and 0.05 per cent, which caused adult mortality of 28.33 and 30.00 per cent, respectively, although no ovicidal action was observed. Similarly, the aqueous fraction at 0.025 and 0.05 per cent resulted in 16.67 and 20.00 per cent adult mortality though the ovicidal action was restricted to 2.67 and 6.67 per cent, respectively. However, the hexane fraction was superior among the different fractions, followed by chloroform fraction (Fig. 3).

Phytochemical analysis of the most effective solvent fractions in this study revealed the presence of three major bio-active compounds *viz*., 4α-phorbol 12,13-didecanoate (4α-PDD), 4-o-methylphorbol 12, 13- didecanoate (4-o-MePDD) and milbemycin b in hexane fraction and two compounds *viz*., 4-o-methylphorbol 12, 13-didecanoate and milbemycin b in the chloroform fraction. Two of these compounds were common to both the fractions. However, 4α-phorbol 12,13-didecanoate is unique to hexane fraction. Phorbol esters are known to affect signal transduction pathways by acting on the biological membranes (Goel *et al.*, 2007). Among the two phorbol esters identified, the role of 4α-phorbol 12,13-didecanoate as a prominent TRPV (Transient receptor potential cation channel) agonist has been widely documented (Alexander *et al*., 2012).

Insecticidal activities of *Jatropha* oil containing phorbol esters have been reported in *Manduca sexta, Helicoverpa armigera, Aphis gossyii, Pectinophora gossypiella, Empoasaca biguttula, Callosobruchus chinensis, Sitophilus zeamays, Phthorimaea operculella, Culex* sp., *Sesamia calamistis, Busseola fusca, Periplaneta americana, Blatella germanica* and *Oncopeltus fasciatus* (Wink et al., 1997). Toxicity of Jatropherol-1, a phorbol based compound, from *Jatropha curcas* on the enzymatic activities and ultra structure of midgut cells in silkworms, *Bombyx mori*, was reported by Jing *et al*. (2005). The phorbol was reported to decrease the protease activities in a time- and dose-dependent manner. *Jatropha* oil has been also reported to have signific-

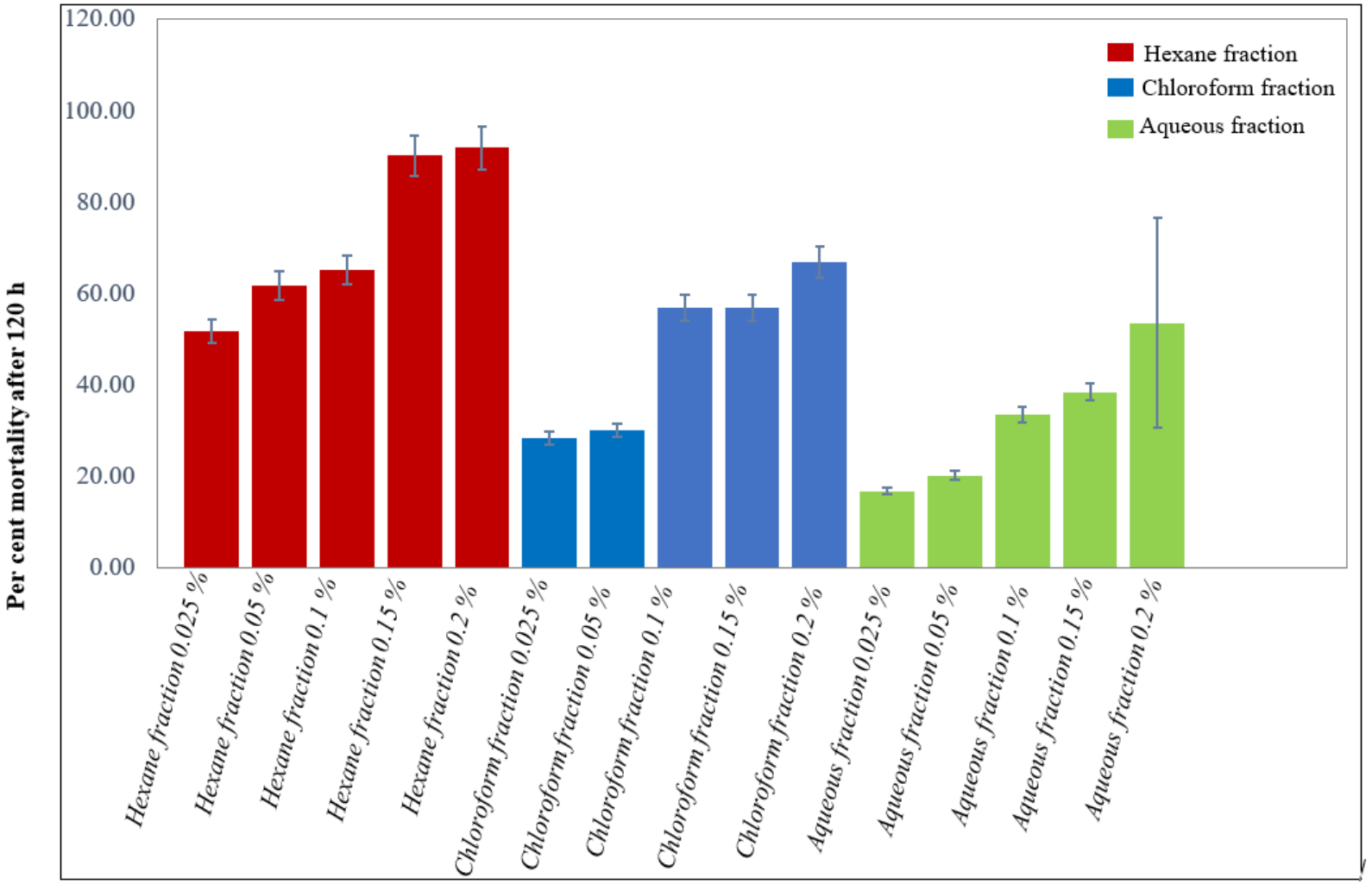

Fig. 3. Effect of solvent fractions of *Tagetes minuta* on gravid females of *Tetranychus truncatus*

ant acaricidal effect on the tick species *Rhipicephalus microplus* (Rizo-Borrego *et al*., 2019). Similarly, milbemycin b, identified in both hexane and chloroform fractions, is a GABA gated chloride channel agonist (Ozoe, 2012), similar to avermectin group of insecticide/acaricide. The acaricidal and insecticidal activities of this compound have been widely documented against ticks and fleas (Bobade, 2019).

Significant adulticidal effects shown by hexane and chloroform fractions of *T. minuta* on the spider mite *T. truncatus* in this study, could be due to the action of these bioactive compounds in the fractions.

Polar fractions of *T. minuta*, extracted using polar solvents such as methanol and water were reported to be composed of various carbohydrates, proteins, phenols, flavonoids, terpenoids and alkaloids (Rikisahedew, 2018). The terpenoid, D-limonene present in the polar fraction of *T. minuta* is known to possess significant insecticidal/acaricidal properties (Karr and Coats, 1988). The adulticidal effect exhibited by the aqueous fraction of *T. minuta* against *T. truncatus* in the present study could be due to these bioactive compounds.

Appreciable adulticidal effect of *T. minuta* as compared to the ovicidal effect against spider mites have been reported in other studies also. Yigezu *et al*. (2022), studied the efficacy of *T. minuta* leaf powder dissolved in water and essential oil against eggs and adults of the two-spotted spider mite *Tetranychus urticae* Koch. At the maximum concentration tested (90 g/L), the leaf extract caused 72 per cent mortality in adult mites after 72 h of treatment, whereas the maximum egg mortality at the same concentration and time after treatment was only 59.25 per cent. Similarly, when essential oil was tested against the adults and eggs, the mortality in each, at 5 per cent concentration of the oil were 92.00 and 83.75 per cent, respectively. Studies in other botanicals have also shown similar trends. When Sarmah *et al.* (2009) tested the efficacy of aqueous plant extract of *Clerodendron infortunatum* against *Oligonychus coffeae*, the extract at 10 per cent concentration recorded cent per cent mortality against adult mites, though ovicidal action was restricted to only 20.58 per cent.

In an earlier study conducted by Laya *et al*. (2021) to evaluate the ovicidal and adulticidal effect of different solvent fractions of *Acorus calamus* on *T. truncatus* in laboratory bioassays, only the methanol fraction of the botanical resulted in significant mortality of eggs and the hexane and chloroform fractions did not show any ovicidal action even at higher concentrations. In contrary to the present findings, in their study, the polar constituents were responsible for ovicidal action. However, similar to the findings of the present study, all the fractions caused appreciable adult mortality in *T. truncatus*.

As the major bioactive compounds identified in the hexane fraction of *T. minuta* act on the nervous system of target organism, the ovicidal activity exhibited at higher doses may not be attributed to these compounds. The treated eggs in this study did not show any signs of deformity indicating that, failure in hatching is not due to the death of the embryo. However, the exact mechanism of appreciable ovicidal effect exhibited only by the hexane fraction of *T. minuta* is not clearly understood, which needs further investigation.

The present study has established the acaricidal properties of *T. minuta* solvent fractions. The efficacy of essential oils extracted from *T. minuta* has already been established by previous studies. Efficacy of essential oils from different plant parts and different growth stages of *T. minuta* were evaluated against *Varroa destructor*, an ectoparasitic mite of *Apis mellifera*. The results of the study showed that the mean percentages of dead mites after six hours of treatment for essential oils extracted from leaves of blooming plants, leaves of non-bloomed plants and flowers, were 97.70, 98.33 and 100.00, respectively (Chamorro *et al*., 2011). Significantly higher adult mortality was also recorded, when a study was carried out to evaluate the efficacy of essential oils from leaves and stems of *T. minuta* against several tick species. The essential oils had over 90 per cent efficacy against four Brazilian tick species *viz., Rhipicephalus microplus*, *Rhipicephalus sanguineus, Amblyomma cajennense* and *Argas miniatus* at a concentration of 20 per cent.

5.1.2. Bioefficacy of *Tagetus minuta* extracts against *Aphis craccivora*

Results of the present study indicated that all the three solvent fractions (hexane, chloroform and aqueous) at five different concentrations (0.025, 0.05, 0.1, 0.15 and 0.2 %) reduced the aphid count by 48-90 per cent, after 5 days of treatment (Fig. 4). On treatment application, the aphids were found to be repelled strongly and moved away from the treated seedlings. No dead adult aphids were noticed on the seedlings. Hence, the reduction in aphid count could primarily be due to the repellent and anti-feedant effects of the botanical fractions. The data showed that there is a sudden decline in aphid count on treated seedlings up to 48 h of treatment, after which the population reduction was less pronounced (Fig. 5). In all the three fractions, at the highest concentration tested (0.2 %), about 60 per cent of the adult aphids were repelled within 24 h of treatment. This could be due to the volatile constituents in the botanical that triggers sudden repellent action in the aphids (Dardouri *et al.*, 2019). Volatile compounds such as D-limonene, dihydro-tagetone, (E)-tagetone, (Z)- tagetone, (Z)-beta-ocimene and allo-ocimene that were previously reported in *T. minuta*, are known to be having repellent properties against insects (Kimutai *et al*., 2015).

Studies have reported the antifeedant properties of the phorbol esters similar to those identified in the bioactive fractions of *T. minuta* in the present study. Phorbol esters extracted from *Jatropha* oil were evaluated for insecticidal activity against *Spodoptera frugiperda* (third instar larvae). At highest concentration of the phorbol esters (0.25 mg ml−1, w/v), food consumption and relative growth rate of *S. frugiperda* larvae were reduced by 33 and 42 per cent, respectively, apart from exhibiting contact toxicity (Devappa *et al*., 2012).

Phoofolo *et al*. (2013) had evaluated the aphidicidal activities of the crude extracts *T. minuta* against the cabbage aphid, *Brevicoryne brassicae*. A comparison was made on lethal and sub-lethal effects of the crude extracts from acetone, methanol, water and a mixture of acetone/methanol/water (7:7:1 v:v). The mixture produced the most toxic extract, followed by methanol and water, whereas acetone produced the least

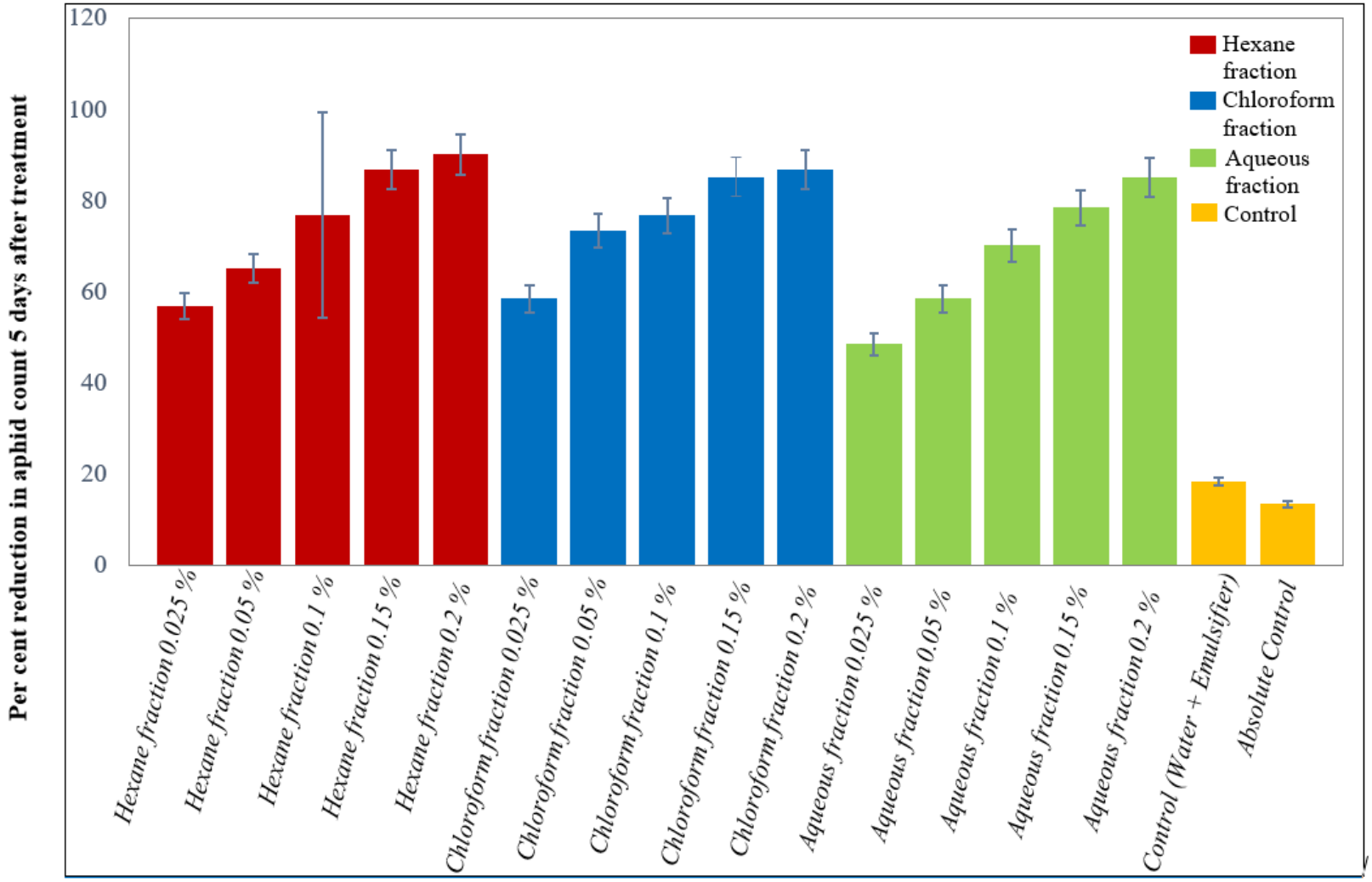

Fig. 4. Effect of solvent fractions of *Tagetes minuta* on adults of *Aphis craccivora*

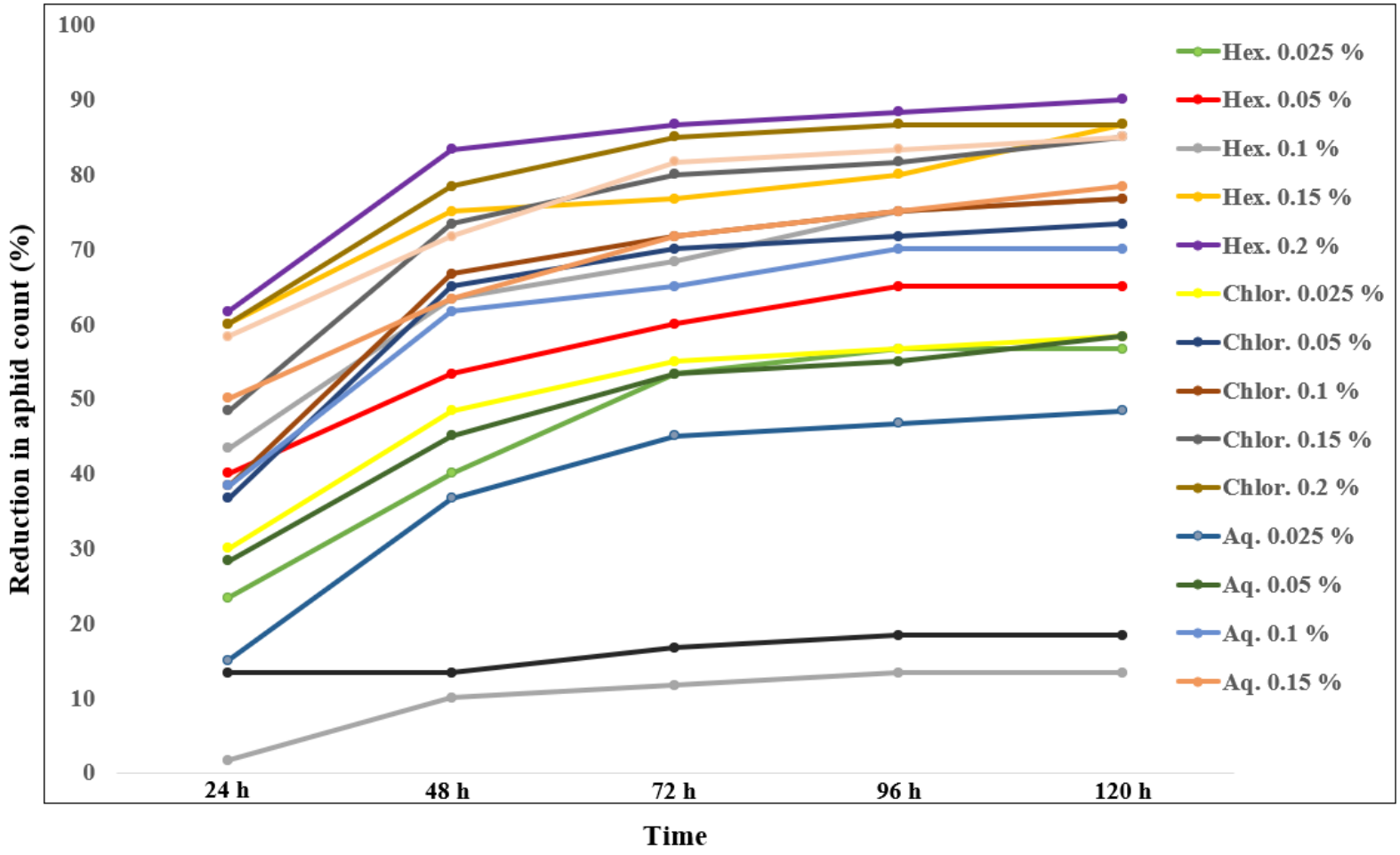

Fig. 5. Effect of solvent fractions of *Tagetes minuta* on adults of *Aphis craccivora* with time

toxic extract. The study also demonstrated that the crude extract of *T. minuta* obtained using water as a solvent is as effective as crude extracts from organic solvent systems in terms of efficacy against cabbage aphids. This observation corresponds to the results of the present study where all the three solvent fractions of the botanical (hexane, chloroform and water) were effective in reducing the aphid population.

Mmbone *et al.* (2014) when tested the crude aqueous extracts of *T. minuta* (150 g/L soaked for 48 hours) and *Tagetes vogelii* (60 g/L soaked for 48 hours) for repellency against the cabbage aphid, *B. brassicae* in the laboratory, the results showed that the aphids were not repelled by any of the extracts used. But in the present study, three different solvent fractions evaluated were found to repel the aphids considerably. This could be due to the difference in the extraction method followed in the two studies. Mmbone *et al*. (2014) used crude aqueous extracts, while in this study highly refined solvent fractions which is expected to possess higher concentration of the bio-active constituents were evaluated.

5.2. Evaluation of botanical extracts against *Tetranychus truncatus* in pot culture

The three botanical fractions of *T. minuta* when tested for efficacy against *T. truncatus* on potted plants of amaranthus in open condition, resulted in more than 75 per cent reduction in mite population. The hexane fraction recorded the highest reduction in mite population (88.78 %) followed by chloroform and aqueous fractions which were on par in their efficacy (77.97 and 76.96 %, respectively). These fractions were found to be on par with neem oil emulsion (2 %) (76.44 % reduction), which is a widely recommended botanical preparation for management of mite pests on different crops. In the present study, laboratory bioassay also indicated a similar trend in efficacy, where hexane fraction was superior over chloroform and aqueous fractions. It exhibited both ovicidal and adulticidal effects against the mite, while, chloroform and aqueous fractions exhibited only adulticidal effect, at higher doses. Hence the higher field efficacy of hexane fraction could be due to the combined effect of ovicidal and adulticidal effects. Though chloroform and aqueous fractions did not record ovicidal

effect in the laboratory, at higher concentrations they exhibited appreciable adulticidal effect. As only the higher concentrations of these fractions, which showed appreciable adulticidal activity in the laboratory, were tested in the field, considerable reduction in field population was recorded in this study (Fig. 6).

The potential of various botanicals, that showed significant mortality of spider mites in the laboratory, in reducing the field populations has been documented in some earlier studies.

The acaricidal activities of the crude aqueous extracts of four botanicals *viz*., *Polygonum hydropiper, Xanthium strumarium, Acorus calamus* and *Clerodendron infortunatum* were investigated under laboratory and field conditions against the tea red spider mite, *Oligonychus coffeae* (Nietner) at two different concentrations (5 % and 10%). In the laboratory, *C. infortunatum* recorded very high mortality of the mite (96 and 100 %) followed by *X. strumarium* (61.5 and 91.8 %), *A. calamus* (57.1 and 88.7%), and *P. hydropiper* (53.9 and 84.2%), 72h after treatment. In the field evaluation also, when the extracts were tested at the above concentrations (5 and 10 %), *C. infortunatum* recorded very high reduction in mite population (81.8 and 100 %) followed by *X. strumarium* (70.9 and 90.2 %), *A. calamus* (57.7 and 86.4 %), and *P. hydropiper* (46.9 and 64.7 %), 14 days after treatment (Sarmah *et al*., 2007).

The aqueous seed extracts of *Melia azedarach*, when evaluated against the tea spider mite, *Oligonychus coffeae* in the laboratory at six different concentrations, showed significant ovicidal and adulticidal effect, which increased with increase in concentration of the extract. The best effective concentrations (6, 8 and 10 %) were tested for their field efficacy that showed highest reduction in population of 95.01 per cent at the highest concentration (10 %), followed by 88.32 (8 %) and 73.35 (6 %) per cent reduction (Roy and Mukhopadhyay, 2012). Roy *et al*. (2014) evaluated the efficacy of the aqueous extract of fruit pericarp of *Terminalia chebula* (1, 2, 3, 4, 5 and 6 %) against *O. coffeae* in the laboratory and the best treatments were tested in the field. The two higher concentrations (5 and 6 %) recorded cent per cent adult mortality and egg mortality of 64.29 and 85. 72 per cent, respectively. In the field, these treatments reduced mite populations by 91.45 and 77.53 per cent; and 96.13 and 95.05 per cent after two and four weeks, respectively.

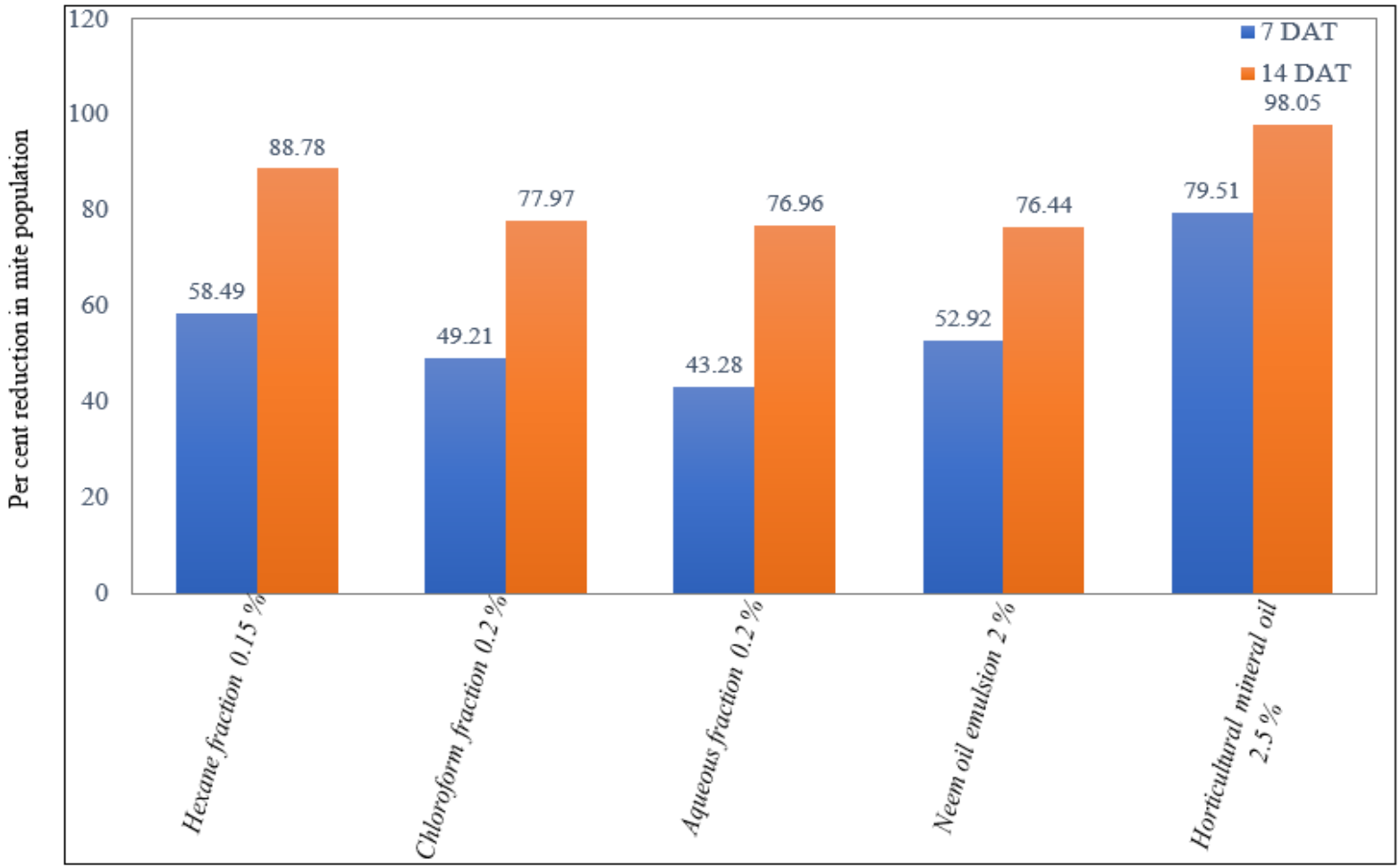

Fig. 6. Per cent reduction of *Tetranychus truncatus* population on amarathus in pot culture

Similarly, Laya (2020) evaluated the best concentrations of the methanol fraction of *Acorus calamus* (0.3 and 0.5 %) and aqueous extract of *Annona squamosa* (7.5 %) (identified in the laboratory bioassay) against *T. truncatus* on cucumber in polyhouse. The study reported cent per cent reduction in mite count in both the concentrations of *Acorus calamus,* while *A. squamosa* recorded 98.81 per cent reduction after 14 days.

5.3. Evaluation of botanical extracts against *Aphis craccivora* in pot culture

The best concentrations of the three solvent fractions of *T. minuta* identified in the laboratory study were evaluated against *A. craccivora* on cowpea, in a pot culture experiment. After 10 days of treatment, all the fractions reduced population of aphids by 68.20 to 78.95 per cent, with the highest reduction recorded by hexane fraction (Fig. 7). In the laboratory study also, hexane fraction showed more efficacy against the aphid.

Volatile compounds present in *T. minuta* viz., D-limonene, dihydro-tagetone, (E)-tagetone, (Z)- tagetone, (Z)-beta-ocimene and allo-ocimene are known to possess repellent properties against insects (Kimutai *et al*., 2015). D-limonene is a major compound in the polar fraction (methanol/aqueous) of *T. minuta* (Rikisahedew, 2018). Phorbol ester and Milbemycin b which are identified in the hexane and chloroform fractions of *T. minuta* can act as nerve poisons by affecting signal transduction pathways (Goel *et al.*, 2007; Ozoe, 2012). The antifeedant property of phorbol esters against insects have also been documented earlier (Devappa *et al*., 2012). There are also reports on the detrimental effects of volatile compounds present in *T. minuta viz.,* β-caryophyllene, limonene and (Z) ocimene on the reproduction of aphids. Tomova *et al.* (2005) reported up to 100 per cent reduction in reproduction in three species of aphids, *viz*., *Acyrthosiphon pisum*, *Myzus persicae* and *Aulacorthum solani*, after five days of exposure to the volatiles of *T. minuta*.

Hence the field efficacy of the *T. minuta* fractions against *A. craccivora* in the present study could be the combined effect of repellency, antifeedancy, neurotoxicity and reproduction inhibition, which needs further investigation.

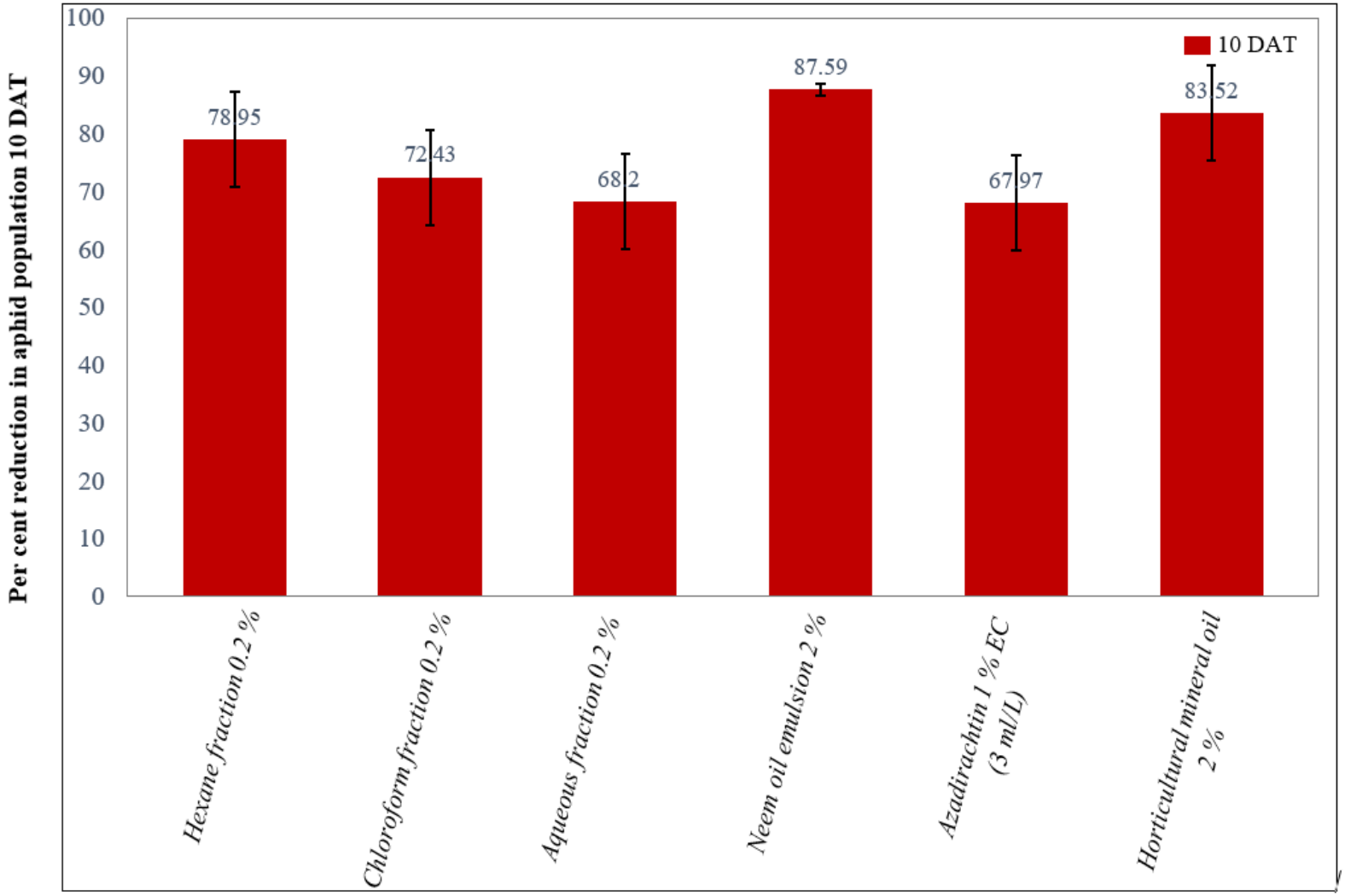

Fig. 7. Per cent reduction of *Aphis craccivora* population on cowpea in pot culture

Field efficacy of *T. minuta* against different species of aphids has been documented from different parts of the world. But these studies evaluated the crude aqueous extracts of the botanical in the field. No studies were conducted to evaluate the solvent fractions of *T. minuta* against aphids.

Ali *et al*. (2010) studied the bio-efficacy of aqueous leaf extracts of *T. minuta* along with three other botanicals *viz., Calotropis procera, Argemone mexicana* and *Azadirachta indica* against the mustard aphid, *Lipaphis erysimi* on Indian mustard, *Brassica juncea*. At higher concentration (1: 2.5 g/ml), *T. minuta* reduced *L. erysimi* population by 96.38 per cent. Kora and Teshome (2016) evaluated the aqueous extracts of chilli, garlic, ginger and Mexican marigold (*T. minuta*) for their insecticidal property against green pea aphid, *Acrythosiphon pisum* in the field. Treatments were applied when aphid population had reached Economic Threshold Level (35% plants infested). The aphid population was reduced to zero in the plants treated with *T. minuta*, chilli and ginger. The above studies had shown that *T. minuta* extracts can reduce aphid population build up considerably.

The present study has proven the acaricidal and insecticidal properties of the Mexican marigold, *T. minuta*. Results of the laboratory and field experiments clearly indicate that the hexane, chloroform and aqueous fractions of aerial parts of the plant possess significant acaricidal and insecticidal properties, with hexane fraction exhibiting superior activity. Hence, the botanical has good potential in exploitation in the management of spider mites and aphids in crop fields. However, studies may be carried out on its biosafety, phyto-toxicity and photo-stability as well as on the formulation of active ingredients so as to exploit the botanical as an effective substitute for chemical pesticides.

Summary

6. SUMMARY

The study entitled "Bioefficacy of *Tagetes minuta* L. against *Tetranychus truncatus* Ehara (Prostigmata: Tetranychidae) and *Aphis craccivora* Koch (Hemiptera: Aphididae)" was conducted in the Department of Agricultural Entomology, College of Agriculture, KAU, Vellanikkara during 2020-2022. The hexane, chloroform and aqueous fractions of *T. minuta* were evaluated for their efficacy against *T. truncatus* and *A. craccivora* in the laboratory, followed by pot culture experiments to evaluate field efficacy. The salient findings of the study are summarized below.

- The dried and pulverized botanical was extracted into respective solvent fractions using hexane, chloroform and water in the increasing order of polarity. The yield of hexane, chloroform and aqueous fractions of *T. minuta* recorded were 3.11, 2.90 and 4.72 per cent, respectively.
- The three solvent fractions of the botanical were evaluated for their ovicidal action against *T. truncatus* at five different concentrations *viz*. 0.025, 0.05,0.1,0.15 and 0.2 per cent following topical bio-assay method. Considerable ovicidal action was exhibited only by the hexane fraction at the higher concentrations of 0.1, 0.15 and 0.2 per cent. Hexane fraction (0.2 %) caused cent per cent egg mortality after 120 h of treatment. Ovicidal action was comparatively less for chloroform and aqueous fractions.
- Efficacy of the solvent fractions against adults of *T. truncatus* was also studied in the laboratory using the concentrations of 0.025, 0.05,0.1,0.15 and 0.2 % against gravid females of the mite by topical bioassay method. The three solvent fractions caused varying degrees of mortality in adult mites in a concentration dependent manner. However, the hexane fraction at 0.2 and 0.15 per cent were significantly superior to all other treatments, causing 91.67 and 90.00 per cent mortality, respectively, after 120 h of treatment. At the same concentration, chloroform fraction (66.67 and 56.67 %) was moderately effective , while the aqueous fraction 53.33 and 38.33 %) showed the least bio-efficacy against adults of *T. truncatus.*

- The bioefficacy of *T. minuta* solvent fractions was evaluated in the laboratory at the concentrations of 0.025, 0.05,0.1,0.15 and 0.2 per cent against *A. craccivora* on cowpea seedlings, following topical application. At the highest concentration, all the fractions recorded significant reduction in aphid count, on par with one another. After 120 h of treatment, the 0.2 per cent concentrations of solvent fractions recorded 90.00, 86.67 and 85.00 per cent reduction in aphid count in hexane, chloroform and aqueous fractions, respectively.
- Qualitative phytochemical analysis of the bio-active fractions *viz.*, hexane and chloroform fractions was carried out by GC-MS/MS and the major bio- active constituents were identified as 4α-phorbol 12,13-didecanoate, 4-o-methylphorbol 12, 13- didecanoate and milbemycin b in hexane fraction and 4-o-methylphorbol 12, 13- didecanoate and milbemycin b in the chloroform fraction.
- Based on the results of the laboratory bioassays (ovicidal and adulticidal bioassays), the best concentrations of the bioactive fractions of *T. minuta* were evaluated for field efficacy against *T. truncatus* in amaranthus in pot culture experiment. Hexane fraction (0.15 %), chloroform fraction (0.2 %) and aqueous fraction (0.2 %) were evaluated along with neem oil emulsion (2%) and Horticultural Mineral Oil (HMO 2.5%). By 14 days of treatment, all the solvent fractions showed considerable field efficacy against *T. truncatus*. Among the different fractions, hexane fraction recorded highest per cent reduction in mite population (88.78 %), followed by chloroform fraction (77.97 %) and aqueous fraction (76.96 %), which were on par with neem oil emulsion. However, HMO exhibited the highest per cent reduction in mite population (98.05 %)
- Field efficacy of the best concentrations of the solvent fractions were evaluated against *A. craccivora* in cowpea plants in pot culture experiment. Hexane fraction (0.2 %), chloroform fraction (0.2 %) and aqueous fraction (0.2 %) were evaluated along with neem oil emulsion (2 %), azadirachtin 1 EC (3 ml/L) and Horticultural Mineral Oil (2 %). Ten days post treatment, among the solvent fractions, hexane fraction resulted in significantly higher reduction in aphid population (78.95 %), followed by chloroform (72.43 %) and aqueous fractions

(68.20 %). However, neem oil emulsion and HMO recorded 87.59 and 83.52 per cent reduction, respectively.

- The study established the excellent acaricidal and insecticidal potentials of the botanical, *T. minuta*. Results of the experiments indicate that hexane, chloroform and aqueous fractions of aerial parts of the plant possess significant acaricidal and insecticidal properties, with hexane showing superior activity. Further, studies may be carried out on its biosafety, phyto-toxicity and photo-stability as well as on the formulation of active ingredients so as to exploit the botanical as an effective substitute for chemical pesticides.

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BIOEFFICACY OF *Tagetes minuta* L. AGAINST *Tetranychus truncatus* EHARA (PROSTIGMATA: TETRANYCHIDAE) AND *Aphis craccivora* KOCH (HEMIPTERA: APHIDIDAE)

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ABSTRACT OF THE THESIS

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ABSTRACT

The spider mite, *Tetranychus truncatus* Ehara and the black cowpea aphid, *Aphis craccivora* Koch are two predominant sucking pests infesting several vegetable crops in Kerala. Farmers depend heavily on chemical pesticides for the management of these pests. However, due to the adverse effects of synthetic pesticides on environment and non-target organisms, there is a growing need for environmentally benign alternatives. This has generated interest in the development of biopesticides, based on botanicals. *Tagetes minuta* L., commonly known as Mexican marigold or wild marigold or southern cone marigold is a plant whose pesticidal properties have been well documented.

The study was carried out in the Department of Agricultural Entomology, College of Agriculture, KAU, Vellanikkara during 2020-2022. The objectives of the study were to identify the bioactive fractions of *T. minuta* and to evaluate the bioefficacy of the solvent fractions against *T. truncatus* and *A. craccivora*. *Tagetes minuta* plants cultivated in the Department of Floriculture and Landscaping, were harvested at the flowering stage followed by shade drying and grinding. The powdered botanical was extracted sequentially using hexane (non-polar), chloroform (medium polar) and water (highly polar) as solvents. The hexane, chloroform and aqueous fractions of *T. minuta* were evaluated for their efficacy against *T. truncatus* and *A. craccivora* in the laboratory at five different concentrations *viz*., 0.025, 0.05, 0.1, 0.15 and 0.2 %.

The ovicidal and adulticidal effects of the solvent fractions were evaluated against *T. truncatus* by topical application method. Considerable ovicidal action was exhibited only by hexane fraction at higher concentrations (0.1, 0.15 and 0.2 %), with cent per cent egg mortality at 0.2 %, after 120 h of treatment. However, all the three solvent fractions caused appreciable mortality of adult mites, in a concentration dependent manner. The hexane fraction at 0.2 % and 0.15 % were significantly superior, causing 91.67 and 90.00 per cent mortality, respectively, followed by 0.2 % chloroform (66.67), 0.15 and 0.10% (56.67), and 0.2 % aqueous fraction (53.33), after 120 h of treatment.

The efficacy of the solvent fractions (0.025, 0.05, 0.1, 0.15 and 0.2 %) against *A. craccivora* was evaluated in the laboratory on cowpea seedlings grown in paper cups. Though the highest reduction in aphid count after 120 h of treatment was recorded in hexane fraction at 0.2 % (90.00 per cent), chloroform (86.67) and aqueous fraction (85.00) also recorded reduction in aphid count on par with it, at the same concentration.

Qualitative phytochemical analysis of the hexane and chloroform fractions was carried out by GC-MS/MS to identify the major bio- active constituents. The two compounds, 4-o-methylphorbol 12, 13- didecanoate and milbemycin b were found in both the fractions, while 4α-phorbol 12,13-didecanoate was found in hexane fraction only.

The best concentrations of the three botanical fractions of *T. minuta* were tested for efficacy against *T. truncatus* on potted plants of amaranthus along with neem oil emulsion (2%) and horticultural mineral oil (2.5%). After 14 days of treatment, the hexane fraction recorded the highest reduction in mite population (88.78 %) followed by chloroform and aqueous fractions which were on par with each other (77.97 and 76.96 %, respectively). Hexane fraction was superior to neem oil emulsion, while chloroform and aqueous fractions were found to be on par with neem oil emulsion (76.44), which is a widely recommended botanical preparation for mite pest management in different crops.

The best concentrations of the three solvent fractions of *T. minuta* identified in the laboratory study were evaluated against *A. craccivora* on cowpea, in a pot culture experiment. By 10^{th} day of treatment, the field efficacy of hexane fraction (78.95 % reduction) was comparable with that of neem oil emulsion (87.59 %), while chloroform (72.43 %) and aqueous fractions (68.20 %) were comparable with azadirachtin 1 EC (67.97 %).

The results of the study indicate that Mexican marigold possess excellent acaricidal and insecticidal properties and that the plant can be explored further, for utilization in the management of spider mites as well as aphids.