EVALUATIONS OF *Moringa oleifera* LAM. AS ACARICIDE AGAINST *Tetranychus urticae* KOCH (Acari: Tetranychidae)

A THESIS

BY

MST. TANJINA AKHTER RUMA

Student No: 1701219

Session: 2022-2023

Thesis Semester: July-December, 2023

MASTER OF SCIENCE (M.S.)

IN

ENTOMOLOGY

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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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DECEMBER, 2023

Dedicated

To

Almighty Allah to Give Me Patience

&

My Beloved Parents, Honourable Teachers and Loving Family

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The Authoress

ABSTRACT

The acaricidal, ovicidal, repellent and oviposition deterrence activity of ethanolic leaf extract from *Moringa oleifera* Lam.were assessed against *Tetranychus urticae.* The study wasconducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU),Dinajpur, at 25 ± 1°C, 65 ± 5% RH and 16:8 (L:D) hours. The effects of ethanolic leaf extract from *M. oleifera*were assessed at 0.5, 1.0, 2.0, 3.0 and 4.0% concentrations. The findings revealed that all concentrations had acaricidal, ovicidal, repellent, and oviposition deterrent effects on *T. urticae*. Adult and nymph mortality rates were increased with increasing doses and time (after 24, 48, and 72 hours). The extracts at 4.0% concentration showed the highest mortality of adults (90.0%) and nymphs (94.0%) after 72 h exposure. Mortality was greater in nymphal stage (56.0-94.0%) than in adult females (7.0-90.0%) when treated with different concentrations. Ovicidal activity (after 7 days) results showed that the lowest egg hatchability was (58.0%) at 4.0% concentration with the highest reduction of viable eggs (-41.00). The LD_{50} values of ethanolic extract for adult females were 3.368, 2.357 and 1.042 at 24, 48 and 72 h, respectively and for nymphs were 0.477, 0.295 and 0.171 at 24, 48 and 72 h, respectively and for eggs it was 8.435 after 7 days. In the repellency test, all concentrations showed repellent effects against *T. urticae* and the highest repellency (92%) was observed at highest concentration (4.0%). Ovipositional deterrence ranged between 9to30%.The result suggested that ethanolic extract from *M. oleifera* leaves can be used as a powerful bio-acaricide for the control of *T. urticae.*

Keywords: *Tetranychus urticae*, *Moringa oleifera*, bioassay, repellency,ovipositional deterrence

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CHAPTER I

INTRODUCTION

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) is one of the most important pest species widely distributed globally. It is a phytophagous mite from the family of Tetranychidae. This species is a highly polyphagous herbivore and a major agricultural pest worldwide that causes hard damage to economics. It is a cosmopolitan species (Lagziri et al., 2015). It presents as a notable pest of field crops, ornamentals, annual and perennial plants (Sim et al., 2003; Seham et al., 2020). It attacks (i)fruit species, including citrus, apple, pear shrubs, and raspberry (Mariethoz et al., 1994), (ii) vegetables, including eggplant, cucumber, bean, okra, and tomato (Gulati, R. 2004; Reddy et al., 2006; Jayasinghe and Mallik 2010; Haque et al., 2011;), (iii) ornamentals, especially dahlia, rose, gerbera, zinnia, and ganda (Silva et al., 2009); and medicinal plants, such as moringa (*Moringa oleifera*), metel (*Datura metel*), alfalfa (*Medicago sativa*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus oflicinalis*), and common vervain (*Verbena oflicinalis*) (Demuth et al., 2007; Abdallah et al., 2019).*T. urticae* causes principal damage, such as defoliation, leaf yellowing, and leaf burning (Abdallah et al.,2019;Youssef et al., 1980), plus the indirect damage by diminishing photosynthesis, transpiration, and a significant decline in yield productivity (Seham et al., 2020;Shaabow et al., 2019).

Throughout the previous few decades, the control of the *T. urticae* population has been dependent mainly on repeated applications of acaricides or pesticides. The biggest problem with this mite is its ability to develop resistancerapidly to the particular acaricide or pesticides used (Badawy et al., 2010). In addition to resistance, the massive and extensive use of conventional pesticides against mites has serious adverse effects on nontarget organisms, human health and the environment (Kumral et al., 2010). To

achieve sustainable management of two-spotted spider mites (TSSM), it is important to diminish the use of conventional acaricides or pesticides and to alternate them with products having a different mode of action (Lee et al., 2003).

The moringa tree, *Moringa oleifera* Lam. (Moringaceae), grows in tropical and subtropical countries and all the tree parts are used for different purposes, including human and animal health, as well as traditional medicine. Different parts of *M. oleifera* have been studied, in particular the leaves, because of its wide range of applications and properties. The literature contains reports stating that moringa leaves have several bioactive compounds such as vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, oxalates and phytates (Leone et al., 2015). Several researchers have reported that *M. oleifera* has pest-control properties. The leaf powder from *M. oleifera* has anti-egg-laying activity over *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) (Anita et al., 2012). Likewise, *M. oleifera* oil has anti-feeding properties over *Spodoptera littoralis* Boisd larvae (Lepidoptera: Noctuidae) (Moawad et al., 2018) and *S. frugiperda* Walker (Lepidoptera: Noctuidae) (Kamel, 2010). Furthermore, the lectins in *M. oleifera* seeds have larvicidal effect over the developing instars of *Aedes aegypti* mosquito L. (Diptera: Culicidae) (Agra-Neto et al., 2014) and over the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Martinez et al., 2012). It has some secondary metabolites (flavonoids, alkaloids and cyanogenic glycosides) which cause insecticidal effects; inhibiting development as well as egg-laying and feeding rates of mites. They can also be repellent and toxic and can have anti-feeding, attractant and killing effects on several species of insects and herbivores (Hikal et al., 2017).

Botanical pesticides are important alternatives to synthetic pesticides since they possess an array of beneficial properties including repellence, antifeedant activity, growth regulatory activity and toxicity to insect and mite pests (Prakash et al., 2008). Moringa can be effectively used as a natural bio-pesticide andthus, it can be included in integrated pest management (IPM) strategies (Adline and Devi, 2014). A significant improvement in pest and disease resistance has also been observed with moringa leaf extract use, with overall yield increases of 20% to 35% (Fuglie, 1999). Considering the bio-pesticides, the present research program was undertaken to assess the effects of ethanolic extract of *M. oleifera* leaves on mortality, egg hatching, repellency and oviposition deterrent of *T. urticae* at different concentrations.

CHAPTER Ⅱ

REVIEW OF LITERATURE

Two-spotted spider sites (TSSM), *Tetranychus urticae* Koch is one of the most important pests in many cropping systems worldwide and the most polyphagous species within the family of Tetranychidae (Leeuwen et al., 2010). TSSM feeding leads to severe leaf damage, reduction in photosynthetic capacity, and finally, leaf abscission (Helle et al., 1985).*T. urticae* is a highly mobile, cosmopolitan species, which is readily spread on the wind, and under optimum conditions, it reaches a high population density, and its presence can cause a reduction in crop yield. One of the most vital strategies for preventing *T. urticae* growth is the application of acaricides.In terms of finding new options for the control of two-spotted spider mites, the use of botanical pesticides (plant extracts) represents a useful tool with less harmful effects on the environment, a low residuality, a slight induction of resistance due to its complex matrix, and with less harmful effects on human health when compared to those of the chemically-synthesized acaricides.Following are some key reviews of the literature that will aid in discussions on the current study project.

2.1 Taxonomy of *T. urticae*

Kingdom: Animalia

Phylum: Arthropoda

Sub-phylum: Chelicerata

Class: Arachnida

Sub-class: Acari

Super-order: Acariformes

Sub-order: Prostigmata

Family: Tetranychidae

Genus: *Tetranychus*

Species: *Tetranychus urticae* Koch

2.2 Seasonal abundance

To develop effective management strategies against any pest it is very important to understand its seasonal activities. The Two-Spotted spider mite, *T. urticae* Koch, (Acari: Tetranychidae), is a globally distributed phytophagous and economically important pest that attack over 1,169 plant hosts, some with economic importance. Wheat, peanut, cotton and ornamentals, as well as vegetables such as pepper, tomato and cucumber are infested by spider mites (Migeon and Dorkeld, 2007; Tehri, 2014).

Seasonal abundance of Two-spotted spider mite is an extremely polyphagous herbivore, feeding on a wide range of host plants throughout the world (Bostanian et al., 2003). It was noted as a major pest of orchid in India (Nagrare and Rampal, 2008) and showed that this mite remains a serious pest of the crop in India, occurring in high densities on the surveyed farms. In general, *T. urticae* incidence occurred throughout the year but was more abundant in most of the orchids in the spring season, with increasing temperature and lower relative humidity during both years.

Rajakumar et al. (2005) who mentioned the lowest populations of *T. urticae* in the winter season on jasmine, which is a valuable ornamental shrub on the Indian subcontinent. The fluctuations in mite population were found to be closely associated with weather factors. Rahaman and Sapra, (1940) observed that an increase in temperature along with a fairly low relative humidity enhances the mite population. High temperature and drought conditions favoured the mite's development (Childers et al., 2007). Gupta et al. (1974) also reported less mite occurrence at low temperatures and high relative humidity. On most of the orchids, *T. urticae* overwintered as diapause in adult stages due to extreme weather during December–January in 2009. The cold

winter climate may not only directly impact the survival of overwintering predaceous mites (Bostanian et al., 2006) but also negatively affect the alternative food source and suitable microclimate (Childers and Enns, 1975).

Common bean *Phaseolus vulgaris* L. is one of the important leguminous crops in many parts of the world including Egypt. The crop in open field conditions has been found to extensively attacked by several arthropod pests, particularly the plant feeder mites of the family Tetranychidae, that are widely spread during the growing season. The wide spread of tetranychid mite species is *T. urticae* which is considered as one of the most serious acarine pests attacking common bean (Latrou et al., 1995 and Razmjou et al., 2009). This spider mite remains active almost all the season and it may reach damaging population levels very rapidly when growing conditions are favourable, resulting significant yield losses (Gregory and Karban, 1998; Gotoh et al., 2004). In Egypt, field observations carried out by Abd-Eaal, (2015) indicated that, the tetranychid mite, *T.urticae* has been reported currently among the most destructive arthropod pests infesting common bean crop. Little is known about factors influencing population dynamics of the spider mite *T. urticae* on this important crop that can be important for development of suitable management programme.

2.3 Crop damage and symptoms of *T. urticae*

The two-spotted spider mite, *T. urticae*, is a polyphagous and prolific pest worldwide that may feed on more than 180 host plants (James and Barbour, 2009). It is one of seriously sucking pests. It feeds on leaves causing damage in chlorophyll and produces white spots that eventually may become more or less coherent (Nachman and Zemek, 2002). It also feeds from the lower epidermis cells by disrupting the leaf tissues to extract the cellular content, resulting in destruction of the individual palisade cells and spongy parenchyma cells (Campbell et al., 1990). As a consequence, the rate of plant

photosynthesis is reduced and desiccation leads to stomatal closure (Freitas et al., 2009).

About 10% host plants infested by *T. urticae* are crops (Vacante, 2016)including economically important crops in different regions of the world (Grbic et al., 2011; Abad et al., 2019). The intensive feeding of mites combined with a rapid increase in population size has a negative effect on the physiology of the whole plant, as well as the yield size and quality (Archer and Bynum, 1993; Suekane et al., 2012). Goftishu et al. (2016)observed the complete destruction of potato plants in fields. Nyoike and Liburd, (2013) reported 50 to 80% loss in strawberry yield, while Jayasinghe and Mallik, (2010) reported up to 50% loss in tomato yield. This high loss in yield has been explained by pest invasion in the early stages of plant development, which was observed in *Cucumis sativus* L. (Cucurbitaceae) (Park and Lee, 2007) cotton (Gore et al., 2013), tomato (Jayasinghe and Mallik, 2010) and potato (Yigezu et al., 2019). Spider mites are also harmful to sugar beet *Beta vulgaris* L. (Chenopodiaceae) grown in Poland (Jakubowska et al., 2018). For several years, large populations of this pest have been observed in plantations in central Poland, especially in Wielkopolskie, Kujawsko-Pomorskie, Łodzkie, Lubelskie, and parts of Mazowieckie provinces. It is a region with a large acreage of sugar beet crops, as well as agrometeorological conditions favourable for the development of the pest during the growing season (dry springs, high summer temperatures 25–30 °C and low precipitation 0−200 mm) (Jakubowska and Fiedler, 2014).

Symptoms of damage caused by mites are initially observed on field margins, and with time they appear in patches all over the field. *T. urticae* causes premature yellowing and drying of the leaves. The feeding of mites that suck out the parenchymal tissue is visible on both sides of the leaf of sugar beet plants. As a result of intense pest feeding,

small, bright spots in a mosaic pattern develop on the upper side of the leaf. The underside of the leaves is covered by a silk web with different developmental stages of the spider mite. The symptoms of early pest feeding are very often underestimated and mistaken for symptoms caused by viruses, nematodes or drought. The increase in two spotted spider mite population and further feeding cause leaf malformation and a web occurs on the plant apex. Plants wilt, turn brown and eventually die back. In the climate of Poland, *T. urticae* might produce from 4 to 6 generations during one growing season. When temperatures are favourable (25–30 °C), which often happens in late spring and summer, a single generation might develop in just 8 days (Jakubowska et al., 2018). The decrease in the root yield caused by intensive feeding of two spotted mite on sugar beet may be from 20 to 50%, and the sugar content in the roots may be reduced by up to 2% (Ulatowska et al., 2015; Jakubowska et al., 2018).

2.4 Plant extracts against *T. urticae*

Use of chemical compounds is an easy and good way for pest control. However, excessive use of pesticides threatens the health of mammals and the residue is perilous for consumers and crops as well as the environment (Lamiri et al., 2001). Nowadays, with regard to the problems arising from the use of chemical pesticides, the tendency has been attended to the use of plant-based pesticides. Insecticide and acaricide activity of plant secondary metabolites or crude plant extracts in particular plant essential oils have been extensively studies in recent decades (Attia et al., 2013; Moharramipour and Negahban, 2014). These compounds have been considered as potential agents because they proved to have a wide range of bioactivity and possess fumigant and contact toxicity, oviposition deterrence and antifeedant as well as repellent effects.

The investigations carried out, which focused on the effects of biopesticides on *T. urticae*,have led to the identification of a large number of plant extracts with acaricidal,

repellent, and deterrent properties. Below are descriptions of some species grouped by plant families whose plant extracts have been used in laboratory studies that have exhibited their biological activity on the two-spotted spider mite.

Amaranthaceae family has aroused interest in different areas such as traditional and alternative medicine, given the properties that have been identified in some of the species that comprise it. Such is the case of *Achyranthes aspera* L., whose secondary metabolites have antinociceptic activity (Barua et al., 2010), or *Chenopodium ambrosioides* Mosyakin et Clemants, which has toxic effects that have been studied in some human parasites (Monzote et al., 2009). Due to these toxic effects, Hiremath et al. (1995) evaluated the acaricidal effect of the extract of this plant. They compared the activity of the methanolic extracts obtained from 21 different species of African plants against *T. urticae* Koch adults using the leaf immersion method. Among the most active extracts, the whole plant of *Celosia trigyna* Linn. exhibited the highest biological activity, causing mortality rates between 40% and 60% of evaluated mites.

Chiasson et al. (2004) also evaluated that an emulsifiable concentrate—obtained from *Chenopodium ambrosioides* Mosyakin et Clemants essential oil extrats at 0.5% produced a mortality of 94.7% on adult *T. urticae*.

Shi et al. (2006) evaluated the effect of *Kochia scoparia* (L.) Schrad extract on *T. urticae* Koch, *T. cinnabarinus* Boisdu-Val, and *T. viennensis* Zacher using three different solvents for extracting: methanol, chloroform, and petroleum ether. The highest mortality of the *T. urticae* Koch was obtained with the chloroform-soluble extract, which exhibited a 78.86% average mortality and an LC_{50} of 0.88 using the dipping method, in which mites were glued to an adhesive tape.

Abbassy et al. (1998) determined the LC_{50} of the alkaloidal extract, the ethanolic extract, and the essential oil of the bulb of the ornamental plant *Pancratium*

maritimum L. (Amaryllidaceae) on the *T. urticae* Koch, whose values were 0.2%, 0.36%, and 1.5%, respectively.

Attia et al. (2011) demonstrated that adult *T. urticae* Koch females to different concentrations of garlic extract (*Allium sativum* L.) and these concentrations ranged between 0.46 and 14.4 mg/L using the Potter Tower application. They determined the LD_{50} and the LD_{90}, whose values were 7.49 and 13.5 mg/L, respectively. On the other hand, they concluded that fecundity was reduced by using the concentrations of 0.36 and 0.74 mg/L. Geng et al. (2014) measured the toxicity by the contact and the repellency of the garlic extract at 20, 10, 5, 2.5, and 1.25 g/L. From these tests, they found that treatment with 20 g/L caused a 76.5% mortality rate on mites at 48 h after its application.

Choi et al. (2004) tested the essential oils of 53 plants to determine their acaricidal potential on *T. urticae* Koch eggs and adults. Among these oils, the highest toxicity was exhibited by species of the family Apiaceae—i.e., *Carum carvi* L. since a 100% mortality rate of adult mites was obtained.

Tsolakis and Ragusa, (2008) studied the effect of a mixture of essential oils from the *C. carvi* L. with potassium salts of fatty acids on the *T. urticae* Koch and one of its predators, *Phytoseiulus persimilis* Athias-Henriot. This combination proved to be very selective, since it generated a mortality rate of 83.4% in *T. urticae* Koch females compared to a 24% mortality rate in *P. persimilis* Athias-Henriot females.

Pavela, (2015) tested acaricidal and ovicidal effects of the methanolic extract of *Ammi visnaga* (L.) Lamarck seeds on *T. urticae* Koch. The efficacy in terms of adult mortality rates increased over time, with LD_{50}s (after 72 h from the time of application) estimated at 17, 10, and 98 $\mu g/cm^2$ for the extract and its two major compounds, khellin and visnagin (furanochromenes), respectively. Moreover, the extract and the two isolated

furanochromenes inhibited the development of eggs and caused their mortality, with LD_{50}s of 13.3, 0.5, and 1.8 μg/cm^2 for the extract, the visnagin, and the khellin, respectively. The application of the extract to leaves infested with *T. urticae* Koch achieved a reduction of the number of individuals in all stages of development.

Derbalah et al. (2013) found that the extract of castor leaves (*Artemisia cinae* O. Berg and C.F. Schmidt ex. Plajakov) exhibited low toxicity against the *T. urticae* Koch, with an LD_{50} of 1326.53 ppm.

Afify et al. (2012) found that the acaricidal activity of *Chamomilla recutita* L. extract on the *T. urticae* Koch and the LD_{50} values obtained for adults and eggs in this study were 0.65% and 1.17%, respectively.

Pontes et al. (2007) studied the acaricidal and the repellent effects of the *Protium bahianum* Daly plant resin oil on the *T. urticae* Koch by fumigant tests and concentrations of the oil (5, 10, 15, 20, and 25 μL, corresponding to 2, 4, 6, 8, and 10 μL/L of air, respectively). They evaluated the fresh resin oil and the old resin oil separately. Results showed that the fumigant effect of the oil in both cases increased with concentration and exposure times and had mortality rates of 79.6% and 59.0% after 72 h for the old and the new resin oils, respectively. Regarding the deterrent effect of oviposition, the fresh resin oil presented an increased activity, with only 14 eggs oviposited at 72 h at a concentration of 10 μL/L of air. In repellency tests, only fresh resin oil showed positive effect against mites.

Hiremath et al. (1995) conducted a study who compared the activity of the methanolic extracts obtained from 21 different species of African plants (Combretaceae)against adults of the *T. urticae* Koch using the leaf immersion method. Among the results found, the *Combretum micronthum* G. Don. and the *Piloitigma vetilicolin* whole plant

extracts demonstrated effects on the rates of *T. urticae* Koch mortality of between 40% and 60%.

Mahmoud et al. (2019) evaluated essential oils from two plants of Cupressaceae family against adult females of *T. urticae* Koch. Oil from leaves of *Cupressus macrocarpa* Hartw.ex Gordon had an LD_{50} of 5.69 µL/L air, whereas *Thuja orientalis* L. leaves resulted in an LD_{50} of 7.51 µL/L air.

Numa et al. (2015) conducted a study in which they tested the susceptibility of *T. urticae* Koch females to the *Cnidoscolus aconitifolius* (Mill) I.M. Johnst. leaf extract using the leaf immersion methodology merged with direct application using an airbrush and a dose of 2000 µg/mL caused a 92% rate of mortality of mite females in the trials.

Rasikari et al. (2005) carried out a screening of the leaf extracts of 67 species of plants belonging to the Lamiaceae family. They were evaluated on the *T. urticae* Koch, which were applied by direct contact with the Potter Tower to bean leaves kept in Petri dishes with cotton. From the extracts tested, 14 had a moderate to acute toxic effect on mites. From these, extracts obtained from the plants *Clerodendrum traceyi* F. Muell., *Premna serratifolia* L.,*Ceratanthus longicornis* (F. Muell.) G. Taylor, *Plectranthus habrophyllus* P.I. Forst, and *Plectranthus* sp. Hann caused a 100% mortality rate, whereas the extracts of *Gmelina leichardtii* F.Muell. & Benth, *Premna acuminata* R. Br., *Viticipremna queenslandica* Munir, *Plectranthus diversus* S.T. Blake, *Plectranthus glabriflorus* P.I. Forst, and *Plectranthus suaveolens* S.T. Blake caused mortality rates that were between 90% and 99%.

Miresmailli et al. (2006) identified the components of *R. officinalis* L. essential oil using GC–MS by column chromatography and tested them individually on the *T. urticae* Koch. In the case of mites reared on bean plants, two compounds revealed a

significant toxicity—1,8-cineol and α-pinene (with 88% ± 4.8% and 32% ± 4.8% mortality, respectively)—whereas for mites raised on tomato plants, the same two compounds were those that revealed a significant toxicity. The resulting values were 80% ± 6.2% and 72% ± 4.8% for 1,8-cineol and α-pinene, respectively.

Çalmaşur et al. (2006) tested the effect of the vapors of three essential oils from *Micromeria fruticosa* L., *Nepeta racemosa* L., and *Origanum vulgare* L. on nymphs and adults of the *T. urticae* Koch and adults of the *Bemisia tabaci* Gennadius, finding the highest mortality rates (96.7%, 95%, and 95%, respectively, for *T. urticae* Koch, and 100% for *B. tabaci* Gennadius) when using doses of 2 μL/L of air at 12 h of exposure.

Brito et al. (2006) evaluated the toxicity of different commercial products based on one of the plants with the highest pesticide potential, the Neem (*Azadirachta indica* A. Juss.). It was tested not only on the *T. urticae* Koch but also on its predators, *Euseius alatus* DeLeon and *Phytoseiulus macropilis* banks at different concentrations (0.25%, 0.5%, and 1.0%) and found that the product had a repellent effect on *T. urticae* Koch and *E. alatus* DeLeo. Additionally, the Neemseto exhibited an important reduction in *T. urticae* Koch fecundity, but on the predatory mites, a significant decrease was only observed when mites were exposed to the highest concentrations. This shows that this product can be a promising option for the management of the two-spotted spider mites within integrated pest management schemes given its relative compatibility with predatory mites.

Araujo et al. (2012) who reported acaricidal and repellent activity of the essential oils obtained from *Piper aduncum* L. leaves and its components separately on the *T. urticae* Koch. The repellent activity was attributed to the components (*E*)-nerolidol, α-

humulene, and β-caryophyllene, while the toxicity was attributed to β-caryophyllene. The extracts and their components exhibited a better performance in fumigation than in contact.

Roh et al. (2011) studied the effect of *Santalum* L. sp. essential oil on the *T. urticae* Koch using the leaf immersion method. Through this methodology, they found that the mortality rate of mites was 87.2% ± 2.9%. Additionally, they noticed an oviposition decrease of 89.3% on leaves treated with oil. Subsequently, they evaluated a mixture of α and β–Sandalool—the two main compounds of *Santalum* L. sp.—on the *T. urticae* Koch and obtained a mortality of 85.5% ± 2.9% and a decrease of 94.7% in fecundity.

Kumral et al. (2010) found that extracts of leaves and seeds of the *Datura stramonium* L. to evaluate their acaricidal, repelling, and deterrent effects on oviposition over *T. urticae* Koch adults at 167.25 mg/L and 145.75 mg/L (for leaves and seeds, respectively) and caused 98% and 25% of the mortality, respectively, for the two concentrations after 48 h of application.

Geng et al. (2014) conducted a study on garlic straw, Allium sativum L. against mite. The contact toxicity and repellent effects of garlic-straw extracts (20, 10, 5, 2.5, and 1.25 g/L) were tested against female adults of *T. urticae* and *T. viennensis* in the laboratory. The 20 g/L concentration caused 76.5% and 54.9% mortality 48 h after treatment on *T. urticae* and *T. viennensis*, respectively.

2.5 Moringa against *T. urticae*

The use of moringa as a bioinsecticide is not only effective against mites; it also offers a less polluting and safer alternative for human health than chemical insecticides. The

leaves and seeds of *M. oleifera* are a source of protein, iron, calcium, ascorbic acid vitamin A and bioactive compounds such as carotenoids, flavonoids, vitamin E and phenolics (Sultana and Anwar, 2008). These bioactive compounds can be useful to pest management strategies. Secondary metabolites (flavonoids, alkaloids and cyanogenic glycosides) have insecticidal effects; inhibiting development as well as egg-laying and feeding rates of mites. They can also be repellent, toxic and can have anti-feeding, attractant and killing effects on several species of insects and herbivores (Hikal et al., 2017). Several researchers have been reported that the ethanolic extract of *M. oleifera* leaf at different concentrations (0.1, 0.5, 1, 5, 10, 15, and 20% (v/v)) against *T. merganser* females treated with 20% (v/v) killed 86.67%, 13.70%, and 96.30% at 24, 48, and 72 h, respectively, as compared to the control treatment (Heinz-Castro et al.,2021). The aqueous extracts of *M. oleifera* seeds at different stages of maturation at a concentration of 20% (w v-1) against *T. urticae*, with higher mortality for green seed extracts (98.18%) followed by mature seed extract (83.22%) (Holtz et al., 2020). Different fractions of *M. peregrina*ethyl acetate fraction had the most acaricidal activity (LC_{50} values with 4.259) on *T. urticae* adults (Seifi et al., 2018). Also, the ethanolic extract of *M. oleifera* leafagainst *O. punicae*Hirst (Acari: Tetranychidae)treated with 0.1 and 20% (v/v) showed mortality of 0.00% and46.67%, 6.67% and 86.67%, 13.70% and 96.67%, at 24, 48 and 72 h, respectively, compared to thecontrol group (Heinz-Castro et al., 2021). In fact, the ethanolic extract of *M. oleifera* leaf at different concentrations (0.1, 0.5, 1, 5, 10, 15, and 20% (v/v)) against *T. merganser* eggs and its residual effect on immature hatched from treated eggs. Eggs treated with 0.1 and 20% (v/v) of the extract showed mortality of 3.11 (0.1% (v/v)) to 72.58% (20% (v/v)), as compared to the control treatment, respectively (Chacon-Hernandez and Heinz-Castro, 2023).

CHAPTER Ⅲ

MATERIALS AND METHODS

3.1 Location

The present study was conducted in the Laboratory of the Department of Entomology, Faculty of Agriculture, Hajee Mohammod Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

3.2 Mite collection and rearing

The adults of *T. urticae* were collected from infested bean plants of HSTU in September 2023. The colonies of mites were cultured on bean plants grown in plastic pots (20 cm D × 20 cm H) at an ambient temperature (25º c) and also maintained in the laboratory in Petri dishes.

3.3 Collection and preparation of the plant material and the extract

Visibly clean leaves of *M. oleifera* were collected from the HSTU campus area. The collected leaves were dried in an oven at 50 ºC for three days, until obtaining a consistent weight. The sample was grounded until forming particles of 1 mm (Castillo et al., 2010). The powder was stored in dark bottles at ambient temperature for the preparation of the extraction. Ethanol solvent was used for preparing the extraction of *M. oleifera.* 1.6 g of *M. oleifera* powder was taken in 15 ml Falcon tubes andadded 10 ml ethanol in different five tubes. Then the tubes were shaken and stirred for 15 minutes with the help of a magnetic stirrer. After that, these were taken in ultrasonic machines for 90 minutes and 70 ºC temperatures. Henceforth, these were centrifuged in centrifuge machines to separate the components of a liquid. Then the mixtures were filtered through a filter paper (Whatman No. 1, 9 mm) into conical flasks. The filtered materials were taken into a conical flask. Serial dilution were used to estimate the

concentration of these mixtures. Finally, the extracts were preserved in tightly corked jars and stored in a refrigerator for experimental use.

3.4 Phytochemical extract analysis

The ethanolic extract of *M. oleifera* was performed to do a qualitative detection test of phytochemicals. The tests included tannins (Gelatine test and 10% NaOH test); saponins (Foam test); flavonoids (Conc. H_2SO_4 test, Lead acetate test, Alkaline test); quinones (Conc. HCL test); Coumarins (NaOH test); phenolic compound (Lead acetate test); alkaloids (iodine test); carbohydrates (starch test).

3.5 Acaricidal effect on adult females and nymphs

The bean leaf discs (3 cm diameter) were cut from the centre of the bean leaves with the help of a sharp-edged cookie cutter. Adult female mites (2 days old) and nymphs were transferred to Petri dishes (9 D × 2 H cm) from the stock culture on leaf discs (4 leaf disc/ Petri dish, 25 adults or nymphs/ disc) facing upside down on wet cotton pads. Leaf discs were sprayed with ethanolic leaf extract of *M. oleifera* at five concentrations (0.5, 1.0, 2.0, 3.0 and 4.0%) with the help of a hand sprayer. Tap water was used in the control. Mortality of mites (nymphs and adult females) were recorded at 24, 48 and 72 hours after treatment. Mites were considered dead if they did not respond to a gentle probe with a fine brush. A stereomicroscope (BST 606, Made in Germany) was used to observe the alive and dead mites (both nymphs and adults).

3.6 Ovicidal effect

The bean leaf discs were used as a substrate for oviposition. Four leaf discs, 3 cm diameter, were used for each treatment and twelve female mites were placed upside down on wet cotton pads in a Petri dish (9 D × 2 H cm) and allowed to lay eggs for

6 hours. After that, the adults were removed and the eggs were checked under a stereomicroscope to ensure that at least 25 eggs were on each leaf disc. The rest of the eggs were destroyed with the help of a sharp pin. The discs were treated with ethanolic leaf extract of *M. oleifera* at four different concentrations (0.5. 1.0, 2.0, 3.0 and 4.0 %) with the help of a hand sprayer. The numbers of hatched and non-hatched eggs were recorded for seven days till hatching with the help of a stereomicroscope (BST 606, Made in Germany).

3.7 Repellency effects on adult females

Leaf discs of bean along with a midrib of 3 cm in diameter were used to evaluate the repellence of the four different concentrations (0.5. 1.0, 2.0, 3.0 and 4.0 %) of *M. oleifera* ethanolic leaf extract. One half portion of the disc was dipped for 10 seconds in the test concentrations while the other half served as the control. The treated discs were air dried for 30 minutes before being placed on water-saturated cotton pads in Petri dishes. Twenty-five adult females (3 days adult) were placed on the midrib of each disc. Each experiment was repeated four times. The number of adult mites present on the treated and untreated halves of the discs was counted 24 hours following mite transformation. The data were presented as percentage repellency (PR) which was determines using the formula described by (McDonald et al., 1970) with some modifications. The formula was follows:

$$PR\ (\%) = (Nc - 50) \times 2$$

Where Nc denotes the percentage of insects found in the untreated half of the leaf disc. Positive (+) values represented repellency whereas negative (-) values represented attractancy. The mean values were then classified according to different classes using the following scale. Present repellency as $>$ 0.01 to $<$ 0.1= class 0; 0.1 to 20= class I;

20.1 to 40= class Ⅱ; 40.1 to 60= class Ⅲ; 60.1 to 80= class Ⅳ; 80.1 to 100= class Ⅴ (McGovern et al., 1977).

3.8 Ovipositional deterrence (OD) and discrimination quotient (DQ)

Ovipositional deterrence was investigated by allowing the adult females (25 individuals/ treatment) to deposit eggs both on the treated and untreated control leaf discs. The degree of deterrence was measured in terms of difference in the number of eggs laid by the females on control and treated leaves. Each treatment was repeated four times. The Discrimination Quotient (DQ) was calculated using the following formula.

DQ = C – T/ C + T

Where, C denotes the number of eggs on control leaves and T represents the number of eggs on treated leaves.

3.9 Statistical analysis

Data were analysed using statistix 10 software and mean separation were done by LSD test. Probit analysis was used to determine LC_{50} and LC_{90} using a GW Basic Software.

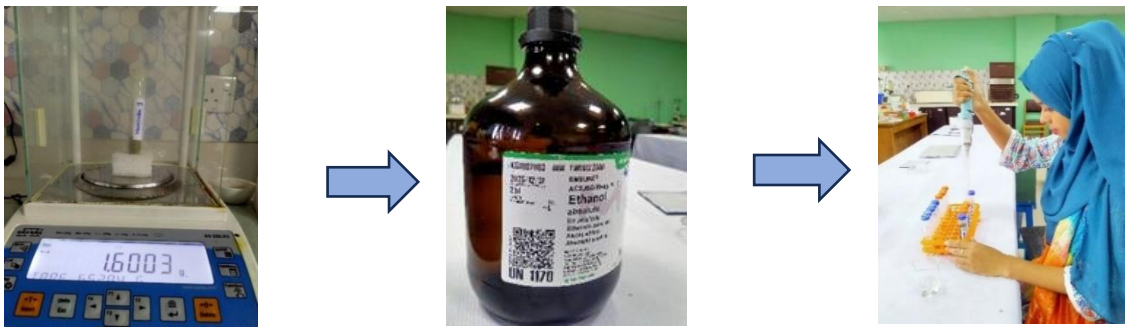
(A) (B)

Plate 1: Rearing of *T. urticae* in plastic pots (A) and Petri dishes(B)

Plate 2: Leaves of *Moringa oleifera* after collecting

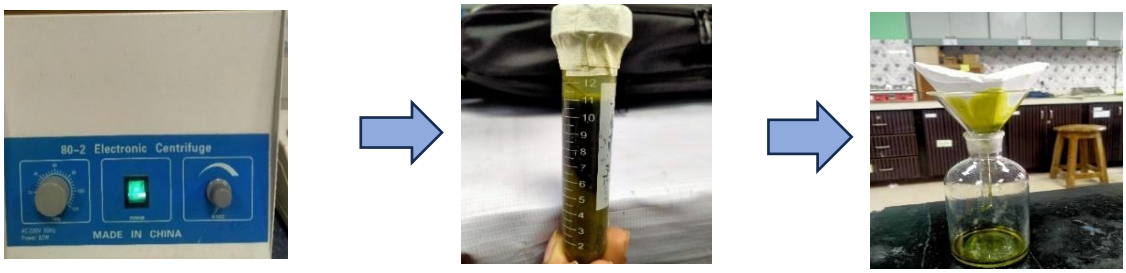
A B

Plate 3: A-Dried leaves (oven condition) and B- powders of *M. oleifera*



AB C

D

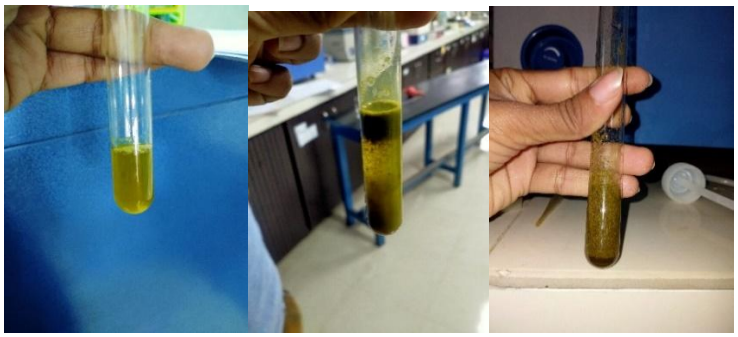


E F G

Plate 4: Preparation (the entire process) of *M. oleifera* extracts (A= weighed in *M. oleifera* powder; B=ethanolic solvent; C= ethanol added with *M. oleifera* powders; D=ultrasonic cleaner to mix the mixture; E= centrifuge machine to separate the components of a liquid; F= separated the liquid form from the solid particles; G= Final extract observed)

Plate 5: Experimental area for treatment of adult females, nymphs and eggs

Plate 6: Stereomicroscope



A B C

Plate 7: Phytochemical extract analysis for Moringa (A= Tannins; B& C= Saponins;)

CHAPTER IV

RESULTS

4.1 Phytochemical

The ethanolic leaf extract of *M. oleifera* has several groups of secondary metabolites, such as phenols, flavonoids, tannins, saponins, quinones and coumarins (Table 1).

4.2 Mortalities of *T. urticae* adults and nymphs

The effects of five concentrations of *M. oleifera*ethanolic leaf extract against adult females and nymphs of TSSM are shown in Tables 1 and 2.At 24, 48 and 72 h following extract application there was a significant effect on the number of TSSM females and nymphs, respectively. At 24 h, mortality ranged of adult females between 7.00% and 61.00%; by 48 h, between 17.00% and 72.00%; by 72 h between 42.00% and 90.00%, compared to the control treatment. Whereas nymphal mortality ranged at 24h, between 56.00% and 87.00%; by 48 h, between 68.00% and 89.00% and by 72 h between 78.00% and 94.00%. The findings revealed that the percentage of *T. urticae* mortality increased as the extract concentration increased.

4.3 Eggs viability

The percentage of eggs hatched at the seventh day was statistically significant. The extracts concentrations of 0.5% and 4.0% caused a residual effect, leading to a reduction of viable eggs, ranging between 13.00% and 41.00%.

4.4 Toxicity of *M. oleifera* leaf extract against *T. urticae* adults, nymphs and eggs

The results of toxicity effect of *M. oleifera* ethanolic leaf extract against *T. urticae* shown in Table 5. In this effect, the mortality of *T. urticae* had diversified noticeably. It was showed a higher mortality at LD_{50} of 1.042 against *T. urticae* adult females at 72 hours after treatment. It was also showed a higher nymphal mortality at LD_{50} value of

0.171 which was more elevated mortality than the adult females. Moreover, in egg phases LD_{50} was observed 8.435 against *T. urticae* after 7 days.

4.5 Repellency

The percent repellency (% PR) of the tested extracts against *T. urticae* was determined and presented in Table 5. Here the repellency rate increased with the increase of doses. Among the concentrations with the strongest repellency effect was showed at 4% concentration (92.00%) followed by 3% (84.00%), 2% (84.00%), 1% (76.00%) and 0.5% concentration (68.00%) after 24 hours of treatment.

4.6 Oviposition deterrent and discrimination quotient (DQ)

Ovipositional deterrence and discrimination quotient value of *M. oleifera* ethanolic leaf extract on *T. urticae* are given in Table 6. Discrimination Quotient (DQ) which has a range from 0.095 to 0.303 is an index for determination of the effect of chemicals on the ovipositional behaviour of mites. When the leaves dipped with different concentrations were provided for egg laying, adult female mites showed some discrimination among the treated leaves concerning the number of eggs laid. Results revealed that the discrimination quotient (DQ) value was high (0.303) at higher concentrations (4%). At 0.5, 1.0, 2.0 and 3.0 % concentrations the DQ value was 0.095, 0.221, 0.287 and 0.298, respectively. And the number of eggs laid per female per day was significantly reduced in all the treated portion of *M. oleifera* extract than the untreated portion.

Table 1. Qualitative phytochemical ("+" = present; "-" = absent) screening of ethanolic extract of *Moringa oleifera* leaves.

Bioactive compound		Test	Bioactive compound		Test
Tannins	+	Gelatine	Quinones	+	Conc. HCL
	+	10% NaOH	Coumarins	+	NaOH
Phenol	+	Lead acetate	Alkaloid	-	Iodine
Saponin	+	Foam	Carbohydrate	-	Starch
Flavonoids	+	Lead acetate			
	+	Conc. H_2SO_4			
	+	Alkaline reagent			

Table 2. Adult mortalityof *T. urticae* of ethanolic extract of *M.oleifera* leaves at different concentrations recorded after spraying (mean ± SE) (%)

Concentration (%)	Adult Mortality (%)(Mean ± SE)		
	24 h	48 h	72 h
0.5	7.00 ± 3.00d	17.00 ± 6.80d	42.00 ± 5.77c
1.0	21.00 ± 5.74bc	35.00 ± 9.98c	57.00 ± 7.72bc
2.0	31.00 ± 5.26c	52.00 ± 2.30bc	70.00 ± 5.03b
3.0	48.00 ± 4.32b	55.00 ± 4.43ab	73.00 ± 7.54b
4.0	61.00 ± 4.43a	72.00 ± 3.65a	90.00 ± 2.00a
Control	5.00 ± 1.0d	9.00 ± 1.91d	14.00 ± 2.0d

Means values and ± standard error (SE) is presented. Different letters in a column indicate significant differences ($p < 0.05$; LSD test).

Table 3. Nymph mortality of *T. urticae* of ethanolic extract of *M. oleifera* leaves at different concentrations recorded after spraying (Mean ± SE) (%)

Concentration (%)	Nymph Mortality (%)(Mean ± SE)		
	24 h	48 h	72 h
0.5	56.00 ± 3.26b	68.00 ± 1.63b	78.00 ± 2.58b
1.0	77.00 ± 3.41a	81.00 ± 3.41a	87.00 ± 3.00ab
2.0	82.00 ± 2.58a	85.00 ± 1.91a	91.00 ± 1.00a
3.0	85.00 ± 2.51a	87.00 ± 1.91a	93.00 ± 1.00a
4.0	87.00 ± 1.91a	89.00 ± 1.00a	94.00 ± 1.15a
Control	18.00 ± 7.74c	29.00 ± 7.37c	35.00 ± 7.37c

The values in a column with a common letter(s) do not differ significantly ($p<0.05$; LSD test)

Table 4. Effect of ethanol extract of *Moringa oleifera* leaves on *T. urticae* eggs hatching (mean ± SE) (%)

Concentration (%)	Hatched eggs	Reduction of viable eggs
0.5	86.00 ± 1.15b	13.00 ± 1.16
1.0	78.00 ± 2.58c	21.00 ± 2.60
2.0	71.00 ± 3.41d	28.00 ± 2.40
3.0	67.00 ± 1.91d	32.00 ± 1.93
4.0	58.00 ± 2.58e	41.00 ± 2.60
Control	99.00 ± 1.00a	-

Values in the same column followed by different letters are significantly different at $p < 0.05$ (LSD test)

Table 5. Statistical comparison of LD_{50}values of *M. oleifera* extract against *T. urticae* adults, nymph and eggs

Phase	Time	LD_{50} (mg/l)	95% confidence level		Regression line	Chi- square, $\chi 2$ (df)
			Lower limit	Upper limit		
Adult	24 h	3.368c	2.862	3.965	Y= 1.635819 + 2.202421X	3.908(3)
	48 h	2.357b	2.008	2.766	Y= 2.539971 + 1.792513X	3.917(3)
	72 h	1.042a	0.855	1.270	Y= 3.424998 + 1.546889X	6.653(3)
Nymph	24 h	0.477a	0.313	0.725	Y= 4.203651 + 1.173312X	3.561(3)
	48 h	0.295a	0.147	0.588	Y= 4.558692 + 0.938795X	0.952(3)
	72 h	0.171a	7.033	0.419	Y= 4.770957 + 0.973841X	0.189 (3)
Egg	7 days	8.435	4.089	17.400	Y= 3.255783 + 0.905568X	0.689 (3)

$\chi 2$ = Goodness of Fit
df = Degrees of freedom
LD_{50} = Median lethal dose
The tabulated value of $\chi 2$ is 7.81 (df = 3)

Table 6. Repellency effect of *M. oleifera* extract on *T. urticae* after 24 h of exposure

Concentration (%)	Repellency (%)	Repellency class
0.5	68	Ⅳ
1.0	76	Ⅳ
2.0	84	Ⅴ
3.0	84	Ⅴ
4.0	92	Ⅴ

Table 7. Effect of *M. oleifera* extract on Oviposition Deterrent of *T. urticae*

Concentration %	No. of eggs laid/female/day	DQ value
0.5	6.58 ± 1.13a	0.095
1.0	6.22 ± 1.08a	0.221
2.0	5.67 ± 1.12a	0.287
3.0	5.65 ± 0.77a	0.298
4.0	5.62 ± 1.06a	0.303
Control	12.17 ± 1.40b	-

Means followed by the same letter do not differ statistically by the LSD ($p \leq 0.05$)

CHAPTER Ⅴ

DISCUSSION

Severe infestation with mite results in economic reduction in the quality and quantity of crop production (Aldosari, 2009). For this reason, many researchers are trying to find out effective natural products to replace synthetic chemicals. All of the plant-derived compounds that have been characterized as having pesticidal activity are plant secondary metabolites (Schmutterer, 1997). The phytochemical evaluation of ethanolic extract of *M. oleifera* leaves showed the presence of secondary metabolites such as (tannin, saponins, flavonoids, quinones, coumarins, phenolic compound). These bioactive substances may be helpful in pest control techniques. Some phytochemicals in the plant, such as flavonoids, alkaloids, glycosides, esters, and fatty acids, affect the behaviour (repelling, deterring/anti-feeding, and alluring) as well as the physiology (toxic, growth retarding, and chemo sterilizing) of several insect and herbivore species (Hikal et al., 2017). These effects are the result of several toxic compounds acting in synergy within the extract (Rincon et al., 2019).

The current research work showed that the ethanolic extract of *M. oleifera* against *T. urticae* adult females and nymphs, revealed as mortality percentage. In case of adults, the highest mortality was (90.00 ± 2.00) at 4.00% concentration after 72 hours and in case of nymphs, the highest mortality was (94.00 ± 1.15) at 4.00% concentration after 72 hours among all the treatments. Several researchers have been reported that the use of plant extracts against *T. urticae* those are similar to current study project. For example, Heinz-Castro et al. (2021) conducted that the ethanolic extract of *M. oleifera* leaf at different concentrations (0.1, 0.5, 1, 5, 10, 15, and 20%) against *T. merganser* females treated with 20% conc. Which showed mortality of 86.67%, 13.70%, and 96.30% at 24, 48, and 72 h, respectively, as compared to the control

treatment. Similarly, Heinz-Castro et al. (2021) also conducted that the ethanolic extract of *M. oleifera* leaf against *O. punicae*(Acari: Tetranychidae) treated with 0.1 and 20% showed mortality of 0.00% and 46.67%, 6.67% and 86.67%, 13.70% and 96.67%, at 24, 48 and 72 h, respectively, compared to the control group. Holtz et al. (2020) revealed that the aqueous extracts of *M. oleifera* seeds at different stages of maturation at a concentration of 20% against *T. urticae*, with higher mortality for green seed extracts (98.18%) followed by mature seed extract (83.22%). Also, several researchers have been reported with different plant extracts against *T. urticae* such as,Pontes et al. (2011) conducted that the ethanol extracts *of Croton rhamnifolius* H.B.K. (Euphorbiaceae) *C. sellowi, C. jacobinensis, and C. micans* had a high mortality on *T. urticae,* whereas *C. sellowi* extract showed the highest effect. Sivira et al. (2011) revealed that the ethanolic extracts of wild oregano and gliricidia leaves were evaluated at different concentrations (5, 10, 15, and 20%) caused 42.2% or 72.5% of mortality to *T. cinnabarinus* (Acari: Tetranychidae) at 10% concentration and maximum mortality (96.6% and 100% caused by wild oregano and gliricidia, respectively) when used at a concentration of 20%. Moreover, Olazaran-Santibañez et al. (2024) conducted that the ethanol extract of *Magnolia alejandrae* (Magnoliales: Magnoliaceae) against *T. merganser* (Acari: Tetranychidae) caused 0.1 and 15% showed mortality of 0.0% and 33.3% at 72 h, respectively, as compared to the control. Also, Vergel et al. (2016) demonstrated that the ethanol extract of leaves of *S. nigrum* on females of *T. urticae* caused at concentrations of 1, 5, 10 and 50 50 µg/mL induced low mortality rates (below 25%) and the highest mortality (85% and 95 %) occurred at 72 h for the highest concentrations of extract applied (600 and 1000 µg/mL).

In this research work, the egg hatching percentage of *T. urticae* was considerably decreased (58.00 ± 2.58) at the highest concentration 4.00% among all the treatments.

Similarly, Chacon-Hernandez and Heinz-Castro, (2023) stated that the ethanolic extract of *M. oleifera* leaf at different concentrations (0.1, 0.5, 1, 5, 10, 15, and 20%) against *T. merganser* eggs and its residual effect on immature hatched from treated eggs. Eggs treated with 0.1 and 20% of the extract showed mortality of 3.11 and 72.58% as compared to the control treatment, respectively.

The present study also demonstrated that the higher acaricidal activity of ethanolic extracts of *M. oleifera* against *T. urticae* with lowest LD_{50} value 1.042 (adults) and LD_{50} value 0.171 (nymphs) after 72 hours whereas the LD_{50} value was 8.435 for eggs after 7 days. Similarly, Afify et al. (2012) indicated that the extracts of chamomile, marjoram and Eucalyptus against *T. urticae* showed the LC_{50} values for adults were 0.65, 1.84 and 2.18, respectively and for eggs 1.17, 6.26 and 7.33, respectively.

The repellency and oviposition deterrent effects were also evaluated in this present study. The results showed that the repellency class of *M. oleifera* extract at different concentration level varied between Ⅰ to Ⅴ and at 4.00% concentration (92%) was found more effective as repellent against *T. urticae* followed by other treatments. In case of oviposition deterrent, the DQ (discrimination quotient) value was showed the highest at 4.00% concentration (0.303) as compared to other treatments. Similarly, Kumral et al. (2010) observed the repellent activities of the ethanol extracts obtained from both leaf and seed in the *D. stramonium* against adult *T. urticae*. El- Sharabasy, (2010) evaluated the potential of crude extracts of *A. Judaica* L. for repellent effect against adult females and immature stage of *T. urticae*. They found that ethanol leaf extraction was more effective as repellent effect females and immature stage of *T. urticae* followed by acetone, petroleum ether and aqueous extraction.Akyazi et al. (2015) observed the toxic and repellent effect of *Prunus laurocerasus* leaves, flower and seed extracts against *T. urticae* and told that at 10% concentration repellent effects

was 96.56%. Moreover, some other researchers have been reported that seed extracts of chinaberry had deterrent effects on the fecundity of *T. urticae* (El-Sawi, 2008, Yanar et al., 2011a, b). Sivira et al. (2011) documented that ethanolic extracts of *Lippia origanoides* H.B.K. (Verbenaceae) and *Gliricidia sepium* (Jacq.) Kunth ex Walp. (Fabaceae) reduced the oviposition of *T. cinnabarinus* Boisduval at 72 h (43.7% and 57.0%) at a concentration of 5% (*v*/*v*), as compared to the control. Similarly, Chacon-Hernandez et al. (2020) found that different concentrations (5, 10, 50, 100, 250, 500, and 1000 µg/mL) of *M. tamaulipana* ethanolic powdered extract have anti-oviposition effects on *T. urticae* females. They reported an oviposition inhibition rate increase between 18.18% to 95.56%, 7.69% to 95.83%, and 11.74% to 95.39%, according to the concentration of the extract, at 24, 48, and 72 h, respectively.

Finally, the results of this research work showed that *M. oleifera* has potential as biopesticide to control of two spotted spider mites. The extract caused chronic toxicity in females, reducing egg viability, repellency effect and the number of eggs laid by *T. urticae* females. Such research might give a clearer idea of the scope of the effects of *M. oleifera* against *T. urticae*. Thus, the present study recommended that plant extracts of *M. oleifera* can beused as a biopesticides against *T. urticae*without harming the beneficial insects and increasing the yield. There need field trial to recommend this product as biopesticides.

CHAPTER Ⅵ

SUMMARY

The current study was conducted to find out the effectiveness of *M. oleifera* leaf extract at different concentrations for their acaricidal, ovicidal, repellency and oviposition deterrent effects against *T. urticae*. Mortality percentage of *T. urticae* adult females showed that the highest mortality indicated at 4.00% concentration (90.00 ± 2.00) after 72 hours among all the treatments whereas 0.5% concentration showed the lowest mortality (42.00 ± 5.77). Nymphal mortality percentage of *T. urticae* also indicated that the highest mortality showed at 4.00% concentration (94.00 ± 1.15) after 72 hours among all the treatments whereas 0.5% concentration showed the lowest mortality (78.00 ± 2.58). The egg hatching percentage was considerably decreased at 4.00% concentration (58.00 ± 2.58). In probit analysis, the lowest LD_{50} value (1.042) indicated the highest toxic effects and the highest LD_{50} value (3.368) indicated the lowest toxic effects against adult females. In case of nymph, the lowest LD_{50} value (0.171) indicated the highest toxic effects and the highest LD_{50} value (0.477) indicated the lowest toxic effect. And in case of eggs, the LD_{50} value was 8.435. The repellency class of *M. oleifera* extract at different concentration level varied between Ⅰ to Ⅴ. At 4.00% concentration (92%) was found more effective as repellent against *T. urticae* followed by other treatments. In oviposition deterrent, the DQ (discrimination quotient) value was showed the highest at 4.00% concentration (0.303) as compared toother treatments.Finally, it was found out that the ethanolic extract of *M. oleifera*had a high-rate of mortality and reduced fecundity against *T. urticae.* Consequently,ethanolicextracts of *M. oleifera*can be most efficient to control *T. urticae* in laboratory condition.

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