

**ACARICIDAL ACTIVITY OF *SYZYGIUM CUMINI* L. FRUIT EXTRACTS AGAINST  
*TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE)**

**A THESIS**

**BY**

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Student No. 1805239

Session: 2018-2019

Thesis Semester: July-December, 2019

**MASTER OF SCIENCE (M.S.)**

**IN**

**ENTOMOLOGY**



**DEPARTMENT OF ENTOMOLOGY**

**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,**

**DINAJPUR**

**DECEMBER 2019**

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HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,  
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**DEPARTMENT OF ENTOMOLOGY  
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DINAJPUR  
DECEMBER 2019**

*Dedicated*

*To*

*Almighty Allah to Give Me Patience and  
Cordage*

*&*

*My Beloved Parents, Honorable Teachers and  
Loving Family*

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The Author

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**ABSTRACT**

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important polyphagous pest that infests many plant species world-wide. The acaricidal, ovicidal and repellent activity of different extracts of *Syzygium cumini* L. fruits were evaluated against *T. urticae* under laboratory conditions in the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. The *S. cumini* fruit extracts were tested at 0.5, 1.0, 2.0 and 3.0% concentration. All the extracts had direct toxic and repellent effects on *T. urticae*. Mortality percentage was gradually increased with the increase of doses. The methanol extract showed the highest mortality (96.87%) of adult females followed by ethanol (91.66%) at 3% concentration. The LC<sub>50</sub> values of methanol, ethanol, acetone and water extracts for adult females were 0.202, 0.281, 0.375 and 1.694, respectively and for eggs were 0.233, 0.255, 0.290 and 2.516, respectively. In the repellency test, all extracts showed repellency effects and significantly decreased the number of eggs on treated bean leaves. The methanol extract was found more effective as repellent against adult females of *T. urticae* followed by ethanol, acetone and water extracts either causing reduction in egg production per female by 96.73, 94.03, 92.50 and 85.0%, respectively. In persistence test, extract of methanol showed highest mortality (41.66%) at 1 hour after treatment followed by ethanol (38.33%), acetone (35.00%) and water (28.33%). The lethal concentration effects of the extracts fade within two or three days. The result suggested that extracts of *S. cumini* has acaricidal activity against *T. urticae*, and the methanol and ethanol extracts are the most efficient.

**Key words:** *Tetranychus urticae*, *Syzygium cumini*, acaricidal activity, repellent effect.

# CONTENTS

CHAPTER	TITLE	PAGE NO.
	<b>ACKNOWLEDGEMENTS</b>	i
	<b>ABSTRACTS</b>	ii
	<b>CONTENTS</b>	iii-iv
	<b>LIST OF TABLES</b>	v
	<b>LIST OF FIGURES</b>	vi
	<b>LIST OF PLATES</b>	vii
<b>CHAPTER I</b>	<b>INTRODUCTION</b>	1-3
<b>CHAPTER II</b>	<b>REVIEW OF LITERATURE</b>	4-12
	2.1 Taxonomy of <i>T. urticae</i>	4
	2.2 Distribution of <i>T. urticae</i>	5
	2.3 Host range of <i>T. urticae</i>	5
	2.4 Crop damage extend of <i>T. urticae</i>	6
	2.5 Damage symptom of <i>T. urticae</i>	7
	2.6 Plant extracts against <i>T. urticae</i>	7
<b>CHAPTER III</b>	<b>MATERIALS AND METHODS</b>	13-18
	3.1 Mite collection and rearing	13
	3.2 Fruits collection	14
	3.3 Chemical reagent	14
	3.4 Preparation of extracts	14
	3.5 Dose preparation	15
	3.6 Acaricidal effect on adult females	16

## CONTENTS (CONT'D)

CHAPTER	TITLE	PAGE NO.
	3.7 Ovicidal effect	17
	3.8 Repellency effect on adult females	17
	3.9 Residual or persistence test	18
	3.10 Statistical analysis	18
<b>CHAPTER IV</b>	<b>RESULTS AND DISCUSSION</b>	19-31
	4.1 Acaricidal effects of different solvent extracts of <i>S. cumini</i> (fruits) against <i>T. urticae</i> adults	19
	4.1.1 Leaf spraying method	19
	4.1.2 Leaf-dipping method	19
	4.2 Ovicidal activity of four different solvent extracts of <i>S. cumini</i> against <i>T. urticae</i>	20
	4.3 Toxicity of four different solvent of <i>S. cumini</i> fruit extracts against <i>T. urticae</i> adults and egg by tropical spray	21
	4.4 Repellency effects of four different solvent of <i>S. cumini</i> fruit extracts against <i>T. urticae</i>	22
	4.5 Residual effects of four different solvent of <i>S. cumini</i> fruit extracts against <i>T. urticae</i>	23
	4.6 Probit regression line	24
<b>CHAPTER V</b>	<b>SUMMARY AND CONCLUSION</b>	32
	<b>REFERENCES</b>	33-49



## LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	Adult mortality of <i>T. urticae</i> by using <i>Syngizium cumini</i> fruit extracts at different concentrations recorded 24 h after spraying (Mean $\pm$ SE) (%)	25
2	Adult mortality of <i>T. urticae</i> by using <i>Syngizium cumini</i> fruit extracts at different concentrations recorded 24 h after dipping (Mean $\pm$ SE) (%)	26
3	Ovicidal effect of <i>Syngizium cumini</i> fruit extracts at different concentrations recorded 7 days after exposure (Mean $\pm$ SE) (%)	27
4	Statistical comparison of LD <sub>50</sub> values of four different <i>Syngizium cumini</i> fruit extracts against <i>T. urticae</i> adults and eggs	28
5	Repellency effects of <i>Syngizium cumini</i> fruit extracts against <i>T. urticae</i> after 24 h of exposure	29

## LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
1	Persistence of <i>Syngizium cumini</i> fruit extracts against <i>Tetranychus urticae</i> at 1, 24, 48 and 72 hours old LD <sub>50</sub> values.	30
2	Relationship between probit mortality and log doses of <i>Syngizium cumini</i> fruit extracts against <i>T. urticae</i> .	31

## LIST OF PLATES

PLATE NO.	TITLE	PAGE NO.
1	a Adult females with eggs of <i>T. urticae</i>	13
	b Protonymph and deutonymphs of <i>T. urticae</i>	
2	Rearing of <i>T. urticae</i> in plastic pots and petri dishes	14
3	Fruits of <i>Syzygium cumuni</i>	14
4	Powders of <i>S. cumuni</i> fruits	15
5	Preparation of crude extracts of <i>S. cumuni</i>	15
6	Percent solution for treatments	16
7	Sprayer used for the experiment	16
8	Treatments of adult females	16
9	Stereomicroscope	16
10	Treatment of eggs	17

# CHAPTER I

## INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch, is the most polyphagous species of spider mites and has been reported more than 1100 host plant species in 140 families of economic value (Pavela, 2017). Both nymphs and adults suck the cell sap from the lower surface of the leaves (Park and Lee, 2002). Feeding can damage protective leaf surfaces, stomata and the palisade layer. Both stomatal and nonstomatal components of photosynthesis were reduced by the injury created by mite feeding (Reddall *et al.*, 2004). It is also produced silk webbing, which is clearly visible at high infestation levels (Jeppson *et al.*, 1975). Leaf yellowing, bronzing, defoliation and plant may die to direct effect of mite (Mersino, 2002). Indirect effects of feeding may include decrease of photosynthesis and transpiration and removing chlorophyll and other cell contents, producing a characteristic yellow speckling on the leaf surface (Badway *et al.*, 2010). For the control of *T. urticae*, the farmers in Bangladesh mostly dependent solely on chemical pesticides because of its quick and easy effectiveness (Endo and Tsurumachi, 2001). However, pesticides used to control the pests and also kill the beneficial insects. This decimation of the natural enemy complex, coupled with high reproductive potential and a short life cycle of the pest mites can lead to the rapid development of outbreaks. Furthermore, control of TSSM has become increasingly difficult due to their resistance to many common synthetic pesticides (Landeros *et al.*, 2002; Uesugi *et al.*, 2002). Two-spotted spider mite has been well-documented to have evolved resistance to over 95 acaricidal or insecticidal active ingredients (Van Leeuwen *et al.* 2010; Grbic *et al.*, 2011). It is therefore important to diminish the use of acaricides or pesticides and to alter them with products having a different mode of action (Choi *et al.*, 2003). Use for natural compounds from plant extracts has been suggested as a viable source of

alternative treatments for insect and mite control because it has no or low toxicity to non-target organisms and mammals, and are less harmful to the environment (Liang *et al.*, 2003). Moreover, numerous plant based pesticides have been reported to have an activities against insects which can delay or prevent resistance development, repellency, feeding and oviposition deterrence, toxicity, and growth regulatory activity (Sing and Saratchandra, 2005; Wang *et al.*, 2007). The use of botanical pesticides can't show resistance capability and also control the mites without hampering the environment.

*Syzygium cumini* L. a very large evergreen tropical tree found throughout the Indian subcontinent belonging to the family Myrtaceae and a genus of 1000 species (Ayyanar and Subash-Babu, 2012; Rafiullah *et al.*, 2006). The plant is also mentioned in literature as Jamun. *Syzygium cumini* is commonly known as jaam, kalo jam in Bengali (Chase and Reveal, 2009). This plant is very well known for their excellent pharmacological properties since ancient age against dysentery and to treat inflammation, diabetes mellitus, constipation, leucorrhoea, stomachalgia, fever, gastropathy, trangury and dermopathy and to inhibit blood discharges in the faces (Bhandary *et al.*, 1995; Shafi *et al.*, 2002). Blackberry shows insecticidal, acaricidal, anti-bacterial, anti-viral, anti-fungal, anti-infective and anti-inflammatory activity (Ziegler *et al.*, 2004; Cichewicz and Rouzi, 2004; Huang *et al.*, 2004; Clercq, 2001). The stem bark is rich in eugenin, fatty acid, ester, quercetin kaempferol, bergenins, flavianoids tannins, pentacyclic triterpenoid betulinic acid, ester of epi-friefelanol, friedelin and a plant sterol  $\beta$ -sitosterol is found in almost all part of plant (Yogeswari and Sriram, 2005; Chaudhary and Mukhopadhyay, 2012). The extracts from leaves, fruit, root-bark and stem-bark showed antifungal activity (Jabeen and Javaid, 2010). The seeds of the tree have also been reported as a rich source of polyphenols, myristic, palmitic, steric, oleic, linoleic, gallic and ellagic acid derivatives, resin, ferulic acid guaicol, resorcinol,

dimethyl ether, corilagin, 3,6-hexahydroxy diphenoyl-glucose, 4,6 hexahydroxy diphenoyl-glucose, 1-galloyl glucose, 3-galloyl glucose and quercetin (Williamson, 2002; Daulatabad *et al.*, 2006). The fruit contains citric acid, anthocyanins, delphinidin-3-gentiobioside, maividin-3-laminaribioside, pentunidin-3-gentiobioside, cyaniding diglycoside pentunidin and maividin (Ravi *et al.*, 2004, Bajpai *et al.*, 2005). Also its fruits have anti-oxidant, anti-cancer, anti-hyperlipidemic, anti-microbial, anti-acaricidal effect (Rabiea *et al.*, 2011; Pareek *et al.*, 2015). Considering the acaricidal properties, this experiment was conducted to detect the performance of *S. cumini* against TMSS. So, the objective of this study was to evaluate the acaricidal activity of *S. cumini* fruit extracts against *T. urticae*.

## CHAPTER II

### REVIEW OF LITERATURE

Mites have always deserved considerable interest because of their small size and especially the amazing habits of some species. The hierrachial classification of mite *T. urticae* is shown below:

Kingdom: Animalia

Phylum: Arthropoda

Sub-phylum: Chelicerata

Class: Arachnida

Sub-class: Acari

Super-order: Acariformes

Order: Prostigmata

Family: Tetranychidae

Genus and species: *Trtranychus urticae* (Koch, 1836)

#### **2.1 Taxonomy of *T. urticae***

*Tetranychus urticae* (common names include red spider mite and two-spotted spider mite) is a species of plant-feeding mite generally considered to be a pest. *Tetranychus urticae* belongs to the phylum Arthropoda, subphylum Chelicerata that is seperated from insects, the class Arachnidae where spiders and ticks also belong, and the other Acarina that is separated from spiders. Its genome was fully sequenced in 2011, and was the first genome sequence from any chelicerate. It falls under the genus *Tetranychus* berlese because the empodium splits distally; usually in 3 pairs of hairs and duplex setae of tarsus I was well separated (Lindquist, 1985).

Koch gave the first denomination *Tetranychus urticae* in his description in 1936. The mite described by Koch was collected in Germany on the stinging nettle *Urtica dioica*. It is known that two forms of *T. urticae*; green and red which are very similar in morphology and widely

distributed (Carbonnelle and Hance, 2004). However the green forms are found in cold and temperate climates, while the form occurs over much of the warmer temperate zone and subtropics (Dupont, 1979).

## **2.2 Distribution of *T. urticae***

*T. urticae* was originally native only to Eurasia, but has acquired a cosmopolitan distribution (Raworth *et al.*, 2002). Two-spotted spider mite originates from temperate climates (Fasulo and Denmark, 2000). It was originally described from European specimens and considered to be a temperate zone species and distributed throughout the tropical and subtropical plants of the world (Jeppson *et al.*, 1975). In Asia the mite was distributed through Bangladesh (Naher *et al.*, 2008), India (Sharma and Pati, 2012), China (Su *et al.*, 2012), Japan (Matsuda *et al.*, 2013) etc.

## **2.3 Host Range of *T. urticae***

The two-spotted spider mite, *T. urticae* is an extremely polyphagous pest which has a huge range of host specificity more than thousand in number. This spider mite reported to attack more than 1100 plant species, belonging to more than 140 different plant families (Takafuji *et al.*, 2000; Migeon and Dorkeld, 2011). It is described as a serious pest of at least 150 economically important agricultural and ornamental plants including corn, cotton, cucumber, beans, tomato, eggplant, peppers and roses (Robertson *et al.*, 2007; Baptiste *et al.*, 2003). The two-spotted spider mite is detrimental pest infesting over 200 species of plants (Lienk *et al.*, 1980). This mite causes considerable damage to eggplant, bean, melon, tomato, strawberry, pumpkin and many other outdoor and greenhouse crops (Chaudhuri *et al.*, 1985; Ahmadi *et al.*, 2007). Host range of TSSM are given below:

**Vegetables:** Cabbage (Si-Jun *et al.*, 2007), Cucumber (Negin *et al.*, 2013), Eggplant (Kumar *et al.*, 2010), Chilli (Weintraub and Palevsky, 2008), Okra and relatives (Kumaran, 2011),



Onion/Garlic/Leek (Greco *et al.*, 2006), Potato (Adango *et al.*, 2006a), Squash/Pumpkin (Abdullah, 2012), Tomato (Maria *et al.*, 2013).

**Fruits:** Apple (Landeros *et al.*, 2013), Banana (Renata *et al.*, 2011), Citrus (Elizabeth *et al.*, 1997), Ficus (Ibrahim and Tulin, 2003), Melon (Negin *et al.*, 2013), Papaya (Karin *et al.*, 2004), Peach (Mobley and Marini, 1990) Pear (Takafuji and Kamibayashi, 1984), Raspberry (Dariusz, 2003), Strawberry (Afifi *et al.*, 2010), Watermelon (Ronaldo *et al.*, 2005).

**Cereals:** Amaranthus (Adango *et al.*, 2006b), Maize/Corn (Gatarayiha, 2010), Shorgum (Collins and Margolies, 1995), Wheat (Renata *et al.*, 2011) etc.

**Flowers:** Rose (Ping-Man So, 1991), Marigold (Ganai *et al.*, 2017).

**Cash Crops:** Cotton (Jimenez, 2014), Jute (Ismail *et al.*, 2007).

A number of vegetable crops and ornamental plants are known to attack by this mite in Bangladesh (Biswas *et al.*, 2004). Outbreaks of *T. urticae* infestation on lady's finger (Okra) and bean in Bangladesh has been reported by (Gapud, 1981).

#### **2.4 Crop damage extend of *T. urticae***

Two-spotted spider mite, *T. urticae* is a major pest on field crops, glasshouse crops, horticultural crops, ornamentals and fruit trees (Van de Vrie *et al.*, 1972). Two-spotted spider mite 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). TSSM 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 tissue desiccation leads to stomatal closure (Freitas *et al.*, 2009). Adult and immature stages of *T. urticae* suck fluid from the lower surface of leaves (Park and Lee, 2002). TSSM feeding causes necrotic spots, leaf

bronzing, and even plant death in severe infestation. An adult TSSM consumes about 6 cells per hour (Bensoussan *et al.*, 2016). Yield losses caused by TSSM feeding approach 15 % for strawberries, 14 % for corn, 14-44 % for cotton, and 23 % for cucumber (Atanassov, 1997; Powell and Lindquist, 1997).

### **2.5 Damage symptom of *T. urticae***

Most of the spider mites feed underside the leaves and typical symptoms of the feeding are small and light colored puncture which, on prolonged exposure, develop into irregularly shaped, white or grayish-colored spots. The colours from yellow to bronze are often characteristics of mite infestation (Tomczyk and Kropczyńska, 1985). Low population density of *T. urticae* on leaves mainly damage the spongy mesophyll tissue and may cause slight injury to the lower parenchyma cell layer. Highest density of *T. urticae* population in the same plant increased the sphere of damage and more severe injury to palisade parenchyma (Sances *et al.*, 1979). The thickness of injured leaves may greatly be reduced, a reduction of thickness in injured bean plant approximately 50% (Mothes and Seitz, 1982). Mite attack decreases the growth rate of leaf area and number of leaves per plant (Avery, 1962; Avery and Briggs, 1968).

### **2.6 Plant extracts against *T. urticae***

Raghavendra *et al.* (2017) conducted an experiment to evaluate the bio-efficacy of plant derivatives and natural oils against *T. urticae*. In these study ten plant derivatives and natural oils was tested. Among them tulsi (*Ocimum sanctum* L.) leaf extract at 10 percent, neem (*Azadirachta indica* A. Juss.) oil at 3 percent and nochi (*Vitex negundo* L.) leaf extract at 5 percent were found to be the best candidates which can be recommended as an alternative to synthetic chemical acaricides for the management of *T. urticae* Koch.

Aslan *et al.* (2004) reported that essential oil vapors from summer savory (*Satureja hortensis* L.) (Lamiaceae) has shown to be effective in controlling motile stages of *T. urticae* in a greenhouse condition.

Calmasur *et al.* (2006) found that three essential oil vapors from hyssop (*Micromeria fruticosa* L.), catmint (*Nepeta racemosa* L.) and Greek oregano (*Origanum vulgare* L.) have been tested for insecticidal and acaricidal efficacy against *T. urticae* and *Bemisia tabaci* Genn. *Tetranychus urticae* adults and/or nymphs mortality rates were the highest (96.7, 95 and 95%) at the highest treatment rate (2 µl/l) for vapor exposure time of 120 hours for *M. fruticosa*, *N. racemosa*, and *O. vulgare* respectively.

Chaisson *et al.* (2004) revealed that an emulsifiable concentrate UDA-245 with 25% Epazote (*Chenopodium. ambrosioides*) essential oil extract (at 0.5%), had a 97.5% mortality on adult *T. urticae*.

Choi *et al.* (2004) found that caraway seed, geranium, lemon eucalyptus, lemongrass, oregano, pennyroyal, peppermint, sage and spearmint caused 100% mortality at a dose of  $19 \times 10^{-3}$  µl /mL air. At  $7.1 \times 10^{-3}$  µl /mL air, lemon eucalyptus essential oil still caused > 85% mortality in *T. urticae*.

Miresmailli and Isman (2006) conducted a study in which rosemary oil was tested on *T. urticae* by painting the leaf disk resulting in an LC<sub>50</sub> of 10.0 µ /liter for adult females on beans and 13.0 µ /liter on tomatoes. 100% mortality of *T. urticae* was achieved with rosemary oil at 20 µ /liter on beans and 40 µ /liter on tomatoes after 24 hours. When constituents of the rosemary essential oil were tested individually, 1,8 cineole and α-pinene were found to be the most toxic to adult female *T. urticae*, although the greatest mortality was achieved with a full mixture of the rosemary constituents.

Benelli *et al.* (2017) found that isofuranodiene and germacrone, isolated from *Smyrniolum olusatrum* essential oil, which were evaluated against *Tetranychus urticae*. Isofuranodiene showed the lowest LD<sub>50</sub> in acute (15.8 lg cm<sup>-2</sup>) and chronic toxicity (11.9 lg cm<sup>-3</sup>). Inhibition of oviposition was found, and IC<sub>50</sub> was 4.1 (isofuranodiene + AgCF<sub>3</sub>SO<sub>3</sub>).

Akyazi *et al.* (2015) revealed that seed extract of *Prunus laurocerasus* at 10% concentration had potential ovicidal and repellency effect on *T. urticae*.

Geng *et al.* (2014) conducted a study on garlic straw *Allium sativum* L. against mite. The ethanol extracts of garlic straw (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.

Numa *et al.* (2015) found that ethanol extract of the leaves of *Cnidocolus aconitifolius* causes a reduction in the number of eggs laid per female per day of *T. urticae*.

Maciel *et al.* (2015) revealed that the ethanolic extract of *Annona muricata* (Annonaceae) seeds showed the highest toxicity to the mite, with LC<sub>50</sub> around 1.77 mg/ml, followed by hexanic and aqueous extracts, with LC<sub>50</sub> estimated at 3.29 and 151.74 mg/ml, respectively. Abamectin caused mortality of 40% to *T. urticae* in a commercial dosage of 100 ml/100 L. The repellent effect of the ethanolic extract, the toxicity on eggs and the residual effect on mites were also evaluated. The concentrations of 0.61, 0.88 and 1.77 mg/ml, as well as abamectin had neutral effects on *T. urticae* and the concentrations of 3.10, 5.11 and 12.07 mg/ml were repellent. The viability of the eggs when sprayed with the ethanolic extract (LC<sub>99</sub>), Abamectin and the control was 9.5, 76.5 and 91.5%, respectively. The residual effect of ethanolic extract was 120 h after application (HAA), with mortality rates above 80%; Abamectin presented residual effect of 48 HAA with 33.3% mortality.

Yanar *et al.* (2011) evaluated that methanol extracts of nine plant species for their ovicidal activity against the two-spotted spider mite *T. urticae* Koch. The greatest mortality was caused by *E. camaldulensis* leaf extract (63.26%), followed by *X. strumarium* fruit (59.64%), *X. strumarium* leaf (57.45%), *S. nigrum* fruit (51.57%), *A. vulgaris* flower (46.80%) and *S. officinalis* seed extract (44.25%). *Lolium perenne* extract (flowers, leaves) caused the least mortality (24.40%). Azadirachtin at 10 g/l concentration was used as a chemical standard and caused 10.09% mortality.

Afify *et al.* (2012) conducted a study on controlling *T. urticae* by extracts of three essential oil from chamomile, marjoram and eucalyptus. 0.5%, 1%, 2%, 3% and 4% concentration were used and chamomile showed the most potential acaricidal efficiency followed by eucalyptus and marjoram. Gas chromatography-mass spectrometer (GC-MS) proved that the major compositions of *Chamomilla recutita* are  $\alpha$ -bisabolol oxide A (35.251%), and trans- $\beta$ -farersene (7.758%), while the main components of *Marjorana hortensis* are terpinene-4-ol (23.860%), *p*-cymene (23.404%) and sabinene (10.904%).

Erdogan and Yilmaz (2017) found that the extract from *Juglans regia* L. (Juglandaceae) in different concentrations (1%, 3%, 6%, 12%) in the leaf dipping method, the 12% concentration of the extract caused the highest mortality of *T. urticae* nymph (90%) and adult (83.00%) stages and the spraying method, the mortality of *T. urticae* adults at the same concentration was 100%.

El-Sharabasy (2010) revealed that the crude extracts of *Artemisia judaica* L. for the toxic and repellent effect against adult females and immature stage of *T. urticae* Koch and its predator *Phytoseiulus persimilis*. And found the ethanolic leaf extraction was more effective as toxic and repellent effect against adult females and immature stage of *T. urticae*, followed by acetone,

petroleum ether and aqueous extraction ( $P < 0.05$ ) and the  $LC_{50}$  values of both adult and immature of *T. urticae* which were 0.29 and 2.97 gm / ml, respectively.

Pavela *et al.* (2016) tested the Mexican sunflower (*Tithonia diversifolia*, Asteraceae) against the two-spotted spider mite *T. urticae* (Tetranychidae) and used two kind of extractions. The methanolic extract  $LD_{50}$  was  $41.3 \mu\text{g cm}^{-3}$  while  $LD_{90}$  was  $98.7 \mu\text{g cm}^{-3}$ . Furthermore, both *T. diversifolia* extracts inhibited oviposition in *T. urticae*. The ethyl acetate extract was the most active oviposition inhibitor, with an  $ED_{50}$  value of  $44.3 \mu\text{g cm}^{-3}$  and an  $ED_{90}$  of  $121.5 \mu\text{g cm}^{-3}$ .

Salman *et al.* (2014) reported that methanolic extracts obtained from sage (*S. officinalis*) and rosemary (*R. officinalis*) plants from the Lamiaceae family in four different concentrations of the plant extracts, which were 1%, 3%, 6% , 12%, were examined against *T. urticae*. The highest death rates of *T. urticae* at nymph and adult stages were found at 12% concentrations of sage and rosemary extracts 79%, 62.2% and 58%, 82.2% respectively.

Rincón *et al.* (2019) reported that the most studied botanical families from the Lamiaceae, the Asteraceae, the Myrtaceae, and the Apiaceae taxons which may be considered as promising elements to be included into integrated pest management for controlling *T. urticae*.

Afify *et al.* (2011) revealed that the ethanolic extracts from *Syzygium cumini* L. had more acaricidal efficiency against *T. urticae*.

Erdogan *et al.* (2012) worked with ethanolic extracts five different plants *Allium sativum* L., *Rhododendron luteum* S., *Helichrysum arenarium* L., *Veratrum album* L. and *Tanacetum parthenium* L. and found high acaricidal efficiency of all plant but no ovicidal effect against *T. urticae*.

Pavela (2016) reported that methanolic extracts of six different medicinal plants viz *A. visnaga*, *G. glabra*, *J. palmata*, *L. carthamoides*, *O. majorana*, *S. officinalis* had acaricidal properties against *T. urticae*.

Hasanuzzaman *et al.* (2015) tested chloroform and methanol extracts of leaf, stem bark, root and seed of *Syzygium cumini* L. and found highest result in case of chloroform extracts.

## CHAPTER III

### MATERIALS AND METHODS

The present study was conducted in the Laboratory of the Department of Entomology, Faculty of Agriculture, Hajee Mohammed Danesh Science and Technology University, (HSTU), Dinajpur, Bangladesh. The experimental periods were November 2018 to May 2019. In an ambient temperature, all experiments were carried out in the laboratory.

#### 3.1 Mite collection and rearing

The adults *T. urticae* were collected from the infested bean plant of Hajee Mohammad Danesh Science and Technology University campus, Dinajpur, Bangladesh in 2018. The colony of mite was cultured on bean plants grown in plastic pots (20 cm d× 20 cm h) and maintained in the laboratory of the Entomology Department, to ensure the continuous supply of mites for the experiment. Some mites also reared on separated bean leaves in Petri dishes (9 D × 2 H cm). Whenever necessary the old leaves were replaced with new leaves in the Petri dishes.

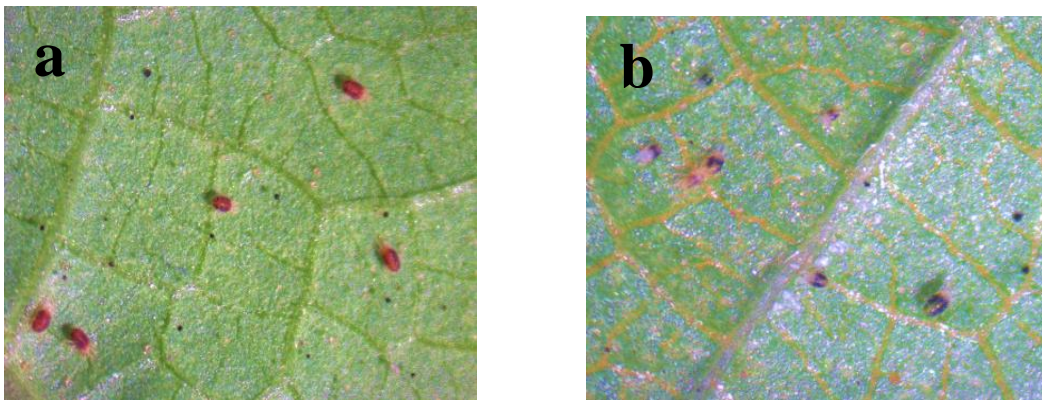


Plate 1: a. Adult females with eggs and b. Protonymph and deutonymphs of *T. urticae*





Plate 2: Rearing of *T. urticae* in plastic pots and petri dishes

### 3.2 Fruits collection

The fully mature ripe fruit of *Syzygium cumini* L. were collected from local market, Bahadur Bazar, Dinajpur, Bangladesh.



Plate 3: Fruits of *Syzygium cumini*

### 3.3 Chemical reagent

All chemicals (methanol, ethanol, acetone, petroleum ether) were collected from (Daejung chemicals and metals Co. Ltd., Korea), Merck KGaA, Germany and Sigma-Aldrich Co.

### 3.4 Preparation of extracts

Collected *S. cumini* fruits were dried under room temperature. Dried fruits finally dried in an oven at 50°C for 1 hours. The dried fruits (both pulp and seed) were grinded and make a fine powder of 80 meshes. Four different solvents (acetone, ethanol, methanol and water) were used for preparig extraction of *S. cumini*. One hundred (100) grams of *S. cumini* powder were taken in a 500 ml beaker and added 300 ml of acetone, ethanol, methanol and water. Then the mixtures were shaken by hand and stirred for 30 minutes with the help of a magnetic stirrer (600 rpm) and

keep them stand for 72 hours. Then the mixtures were filtered through a filter paper (Whatman No. 1, 9 mm) into the conical flasks. The filtrated materials were taken into a conical flask and evaporated the filtration with the help of a rotary evaporator at 65°C for acetone, 80°C for ethanol, 70°C for methanol and 102°C for water. Finally, the semisolid crude extracts were preserved in the tightly corked glass vials and stored in a refrigerator at 3°C for experimental use.



Plate 4: Powders of *S. cumini* fruits



Plate 5: Preperation of crude extracts of *S. cumini*

### 3.5 Dose preparation

The crude extracts were weighted in the electronic balance and dissolved in ethanol, methanol acetone and water solvent for making different concentrations. Prior to conducting the study, a pilot experiment was done to obtain the appropriate dose in a different studies.



Plate 6: Percent solution for treatment



Plate 7: Sprayer used for the experiment

### 3.6 Acaricidal effect on adult females

Three centimeter diameter leaf discs were cut from the center of bean leaves with the help of a sharp edged cookie cutter. Adult females mites (3 days old) were transferred to Petri dishes (9 D × 2 H cm) from the stock culture on leaf discs (4 leaf discs /Petri dish, 25 adults/disc) facing upside down on wet cotton pads. Leaf disc were sprayed with the help of a hand sprayer and dipped with the help of a rubber covered forceps with four different solvent extracts of *S. cumini* at four different concentrations (0.5, 1.0, 2.0 and 3.0%). General tap water was used in the



Plate 8: Treatment of adult females



Plate 9: Stereomicroscope

control. Mortality was recorded at 24 hours after treatment. Mites were considered dead if they were not respond to a gentle probe with a fine brush. A stereomicroscope (BST 606, Made in

Germany) was used in this experiment to observe the alive and dead mite. Mortality percentage was corrected by using Abbott's corrected formula (Abbott, 1925).

### 3.7 Ovicidal Effect

The bean leaf discs were used as a substrate to oviposition. Four leaf discs, 3 cm diameter, were used for each treatment and fifteen 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 5 hours. After then, the adults were removed and the eggs were checked under a stereomicroscope to ensure that at least 25 eggs on each leaf disc. The rest of the eggs were destroyed with the help of a sharp pin. The discs were treated with four different concentrations (0.5, 1.0, 2.0 and 3.0%) of four different solvent extracts of *S. cumini* 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).



Plate 10: Treatment of eggs

### 3.8 Repellency effect 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 solvent extracts of *S. cumini* mixture. One-half portion of the disc was dipped for 10 seconds in the test concentrations and the other half was used as a control. The treated discs were allowed to dry in the open air for 30 minutes and placed on water saturated cotton pad in Petri dishes. Twenty five adult females (3 days adult) were put on the midrib of

each disc. Each experiment was replicated four times. The number of adult mites present on the treated half and untreated half of the discs was recorded 24 hours after mite transformation. The number of egg laying on each side of leaf discs was also recorded under a stereomicroscope. The data were expressed as percentage repellency (PR) which was calculated by using the formula described by (McDonald *et al.*,1970) with some modifications. The formula was following:

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

Where  $N_c$  represents the percentage of insects present in the untreated half of the leaf disc. Positive (+) values expressed repellency and negative (-) values attractancy. The mean values were then categorized according to different class using the following scale. Present repellency > 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).

### **3.10 Residual or persistence test**

Solvent extracts of *S. cumini* fruits were applied on bean leaf disc (3 cm diameter) according to  $LC_{50}$  dose with a hand sprayer. Fifteen (15) adult females of *T. urticae* were carefully exposed to 1, 24, 48 and 72 h old residue with the help of a fine camel hair brush. Every treatment was replicated 4 times. The data were recorded after 24 hours of each released of mite into the leaf discs. Mites were considered dead when they failed to move even after gently probed with a fine brush.

### **3.11 Statistical analysis**

Data were analyzed with one way ANOVA followed by Tukey HSD test at  $p < 0.05$ , using SPSS software (2007). Probit analysis was used to determine  $LD_{50}$  using a software developed in the Department of Agricultural and Environmental Science, University of Newcastle Upon Tyne, United Kingdom (Finney, 1947 ; Busvine, 1971). The program also calculates the limits of  $LD_{50}$  and the heterogeneity at 5% level of confidence is tested by chi square value.

## **CHAPTER IV**

### **RESULTS AND DISCUSSION**

#### **4.1 Acaricidal effects of different solvent extracts of *S. cumini* (fruits) against *T. urticae* adults**

##### **4.1.1. Leaf spraying methods**

The acaricidal activity of four different solvent extracts of *S. cumini* (fruits) at a concentration of 0.5, 1.0, 2.0, and 3.0% against the adult female of *T. urticae* were shown in Table 1. The result showed that methanol extract, had the most efficient acaricidal activity against *T. urticae* followed by ethanol and acetone extracts. At 0.50% concentration, methanol extracts showed significantly higher acaricidal activity over control and all other treatments. At 3.0% concentration mortalities in the four solvent extract treatments increased considerably compared to those at 0.50, 1.0 and 2.0% concentration and methanol solvent extract was showed the highest adult mortality. At 3.0% concentration methanol solvent extract achieved 96.87% mortality, followed by ethanol (91.66%), acetone (71.87%) and water (62.50%), respectively but no significant differences were observed between methanol and ethanol solvent extracts (Table 1).

##### **4.1.2. Leaf-dipping method**

In the leaf dipping method, it was observed that methanol extract had significant mortality on *T. urticae* adult. In all the treatments, the highest effect occurred at a concentration of 3.0% while the smallest effect was at 0.5%. The increased concentration led to increasing the adult mortality. Statistical analysis ( $P < 0.05$ ) showed that the methanolic fruits extracts of *S. cumini* exhibit the highest mortality of *T. urticae* adults. The lowest mortality was at the extract of water. The

methanolic solvent extract of *S. cumini* was found to have good contact acaricidal activity against the *T. urticae*. Yanar *et al.* (2011) demonstrated that methanol extracts of *Melia azedarach* fruits were effective against adults of *T. urticae* and showed contact and residual toxicity effects after 24 hours (76.45 and 74.57% mortality, respectively). Afify *et al.* (2011) investigate the acaricidal activity of different extracts (ethanol, hexane, ether ethanol ethyl acetate and water) of *S. cumini* against *T. urticae*. They found that ethanol extract showed a higher mortality rate of female 24 hour after spraying. But in the present study ethanol extract displayed the second highest mortality against *T. urticae*. Erdogan and Yilmaz (2017) inspect methanol extraction of *Juglans regia* at four different concentrations (1%, 3%, 6% and 12%) against *T. urticae* and found that the mortality rate was higher in the spraying method (72%, 88%, 90% and 100%) than the dipping method (57%, 65%, 65% and 83%) respectively. Almost similar results were found in the present study. Moussa *et al.* (2010) reported that methanol extracts of *Cassia sp.* and *Acacia saligna* (Labill.) leaves demonstrated acaricidal effects against *T. urticae*.

#### **4.2 Ovicidal activity of four different solvent extracts of *S. cumini* against *T. urticae***

Ovicidal activities of four different fruits solvent extracts of *S. cumini* are presented in Table 3. All the treatments showed significant mortality of eggs over control. At 0.50% concentration ethanol extract showed higher mortality of eggs which is significantly different from other extracts at 1.0 and 2.0% concentration and control but no significant difference observed between ethanol and methanol extracts. At 3.0% concentration ethanol extract reached 95.0% mortality followed by methanol (92.0%), acetone (74.0%) and water (57.0%) extracts, respectively. The ovicidal activities of ethanol and methanol extracts of the *S. cumini* have a

good effect on *T. urticae*. Kumral *et al.* (2009) reported that ethanol extracts of *D. stramonium* leaves and seeds exhibited acaricidal, oviposition deterrent activities against *T. urticae*. Salman *et al.* (2014) investigate the methanol extracts of sage (*S. officinalis*) and rosemary (*R. officinalis*) against *T. urticae* egg and found that ovicidal activity in rosemary extract was 82.2% at 12% concentration. Akyazi *et al.* (2015) opined that ethanolic extracts from leaves, flower and seed of *Prunus laurocerasus* had the highest effect against *T. urticae* eggs and mortality was 96.56% at 10% concentration. The egg mortality of *T. urticae* using the methanol extract of river red gum *Eucalyptus camaldulensis* leaves was 63.26% (Yanar *et al.*, 2011). In another study, Ghaderi *et al.* (2013) observed that the ovicidal activity of methanolic extracts of *S. meifolia*, *A. orientale*, *T. elliptica* and *P. viscosa* against *T. urticae* eggs were 45.84, 41.40, 40.11 and 37.66 % respectively. Auamcharoen *et al.* (2015) stated that crude methanol extracts of *D. grandiflora* extracts showed moderate repellency and also inhibited egg production in this mite species.

#### **4.3 Toxicity of four different solvent extracts of *S. cumini* to *T. urticae* adults and eggs by topical spray**

Methanol extracts of *S. cuminin* fruits showed the highest adulticidal properties followed by ethanol, acetone and water extracts (Table 4). The LC<sub>50</sub> values after 24 hours for adults were 0.202, 0.281, 0.375 and 1.694, respectively. On the other hand, ethanol extracts showed the highest egg mortality followed by methanol, acetone and water extracts, respectively. The LC<sub>50</sub> values for eggs were recorded 0.233, 0.255, 0.290 and 2.516, respectively after 7 days. From the probit analysis, it was observed that all the tested extracts were more or less effective for controlling *T. urticae* but ethanol and methanol extracts were the most effective. Pavela *et al.* (2016) reported that the LD<sub>50</sub> value of methanol extracts of mexican sunflower *Tithonia*



*diversifolia* against *T. urticae* were 41.3 mg cm<sup>-3</sup> and LD<sub>90</sub> of 98.7 mg cm<sup>-3</sup> and the LD<sub>50</sub> value of ethyl acetate extraction of *T. diversifolia* against *T. urticae* were 83.5 mg cm<sup>-3</sup> and LD<sub>90</sub> of 153.5 mg cm<sup>-3</sup>. Pavela (2015a) also observed the ovicidal efficacy of the methanol extract of *Ammi visnaga* seeds against *T. urticae* and found the LD<sub>50</sub> value for egg mortality was 13.3, 0.5 and 1.8 mg cm<sup>-2</sup> respectively.

#### **4.4 Repellent effects of four different solvent extracts of *S. cumini* fruits against *T. urticae***

Percent repellency (%PR) for the tested extracts against *T. urticae* was calculated and presented in Table 4. Here the repellency rate increased with the increase of doses. Among the extracts, ethanol showed the highest repellency effect (96.00%) followed by methanol (94.00%), acetone (74.00%), water (58.00%) at 3% concentration after 24 hours of treatment. The number of eggs laid by females showed a significant reduction as compared to control. For egg laying methanol and ethanol extracts showed significant reduction over control and water. There is no significant difference observed among methanol, ethanol and acetone extracts respectively. Akyazi *et al.* (2015) observed the toxic and repellent effect of *Prunus laurocerasus* leaves, flower and seed extracts against *Tetranychus urticae* and told that at 5 10% concentration repellent effects was 96.56 %. Saber (2004) also stated that in *Artemisia monosperma* Del. (Asteraceae) had repellency effects against females of *T. urticae*. Antonious *et al.* (2006) opined that methanol extracts from accessions PI-596057 (*Capsicum baccatum*L.), PI-195299 (*C. annuum*L.), and Grif- 9270 (*C. annuum*) (Solanaceae) may have a great potential for repelling against *T. urticae*. 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 against adult females and immature stage of *T. urticae*, followed by acetone, petroleum ether and aqueous extraction. Hasanuzzaman *et al.* (2015) tested with n-hexane, acetone, chloroform and methanol extracts of leaf, stem bark, root and seed of *S. cumini* and found that methanol extracts of seed showed the higher repellency against *C. chinensis*.

#### **4.5 Persistence effects of four different solvent of *S. cumini* fruit extracts against *T. urticae***

The results of persistence effect of different solvent extracts of *S. cumini* was presented in Figure 1. Control treatment did not show any mortality. In the persistence test, the mortality of *T. urticae* varied noticeably. One hour after treatment methanol extracts showed highest mortality (41.66%) followed by ethanol (38.33%) acetone (35.00%) and water (28.33%). At 24, 48 and 72 hours after treatment all *S. cumini* fruits extract showed significantly different mortality rates than the untreated control except water extracts. Methanol extracts showed a higher mortality rate than all other treatments. But there is no significant difference was observed between ethanol and acetone extracts. However, methanol extracts (fresh) showed the highest mortality (41.66%) at LC<sub>50</sub> value of 0.202 at fresh treatment and water extract showed the lowest mortality (28.33%) at LC<sub>50</sub> value of 1.69 against *T. urticae* adult females at 72 hour after treatment. Yanar *et al.* (2011) demonstrated that methanol extracts of *Melia azedarach* fruits were effective against adults of *T. urticae* and showed contact and residual toxicity effects after 24 hour (76.45 and 74.57% mortality, respectively). Mousa and EL-Sisi (2001) indicated that cotton seed oil was effective in its initial and residual effects against eggs of *T. urticae* on squash crop. Saied *et al.* (2002) also they found that super masrona caused a high residual effect (87.61%) against *T. urticae* population in cotton crops.

#### 4.6 Probit regression line

The probit regression line of four different extracts of *S. cumini* fruit extracts against *T. urticae* at 24 hours are presented in the Figure 2. The adult mortality rates showed positive correlation with the dose of all of the treatments. The probit regression line of *S. cumini* fruit extracts of three solvents showed a clear linear relationship between probit mortality and log doses. The calculated probit regression equation of different extracts of *S. cumini* fruit against *T. urticae* after 24 hour were  $Y= 4.566181 + 1.420348X$  for methanol,  $Y= 4.481631 + 1.152194X$  for ethanol,  $Y= 4.682611 + 0.552773X$  for acetone and  $Y= 3.472274 + 1.24292X$  for water respectively. The methanol extracts Showed highest mortality followed by ethanol, acetone and water (Figure 2).

Table 1. Adult mortality of *Syngizium cumini* fruit extracts at different concentrations recorded 24h after spraying (Mean  $\pm$  SE) (%)

Treatments	Adult mortality			
	0.5% Conc.	1.0% Conc.	2.0% Conc.	3.0% Conc.
Acetone	55.00 $\pm$ 1.00c (53.12)	61.00 $\pm$ 2.51c (59.37)	64.00 $\pm$ 1.63c (62.50)	73.00 $\pm$ 1.91b (71.87)
Ethanol	64.00 $\pm$ 1.15b (62.50)	72.00 $\pm$ 1.63b (70.83)	80.00 $\pm$ 2.82b (79.16)	92.00 $\pm$ 1.63a (91.66)
Methanol	74.00 $\pm$ 1.15a (72.91)	85.00 $\pm$ 1.91a (84.37)	90.00 $\pm$ 2.00a (89.58)	97.00 $\pm$ 1.00a (96.87)
Water	28.00 $\pm$ 1.63d (25.00)	42.00 $\pm$ 2.58d (39.58)	55.00 $\pm$ 1.91d (53.12)	64.00 $\pm$ 1.63c (62.50)
Control	4.00 $\pm$ 1.63e	4.00 $\pm$ 1.63e	4.00 $\pm$ 1.63e	4.00 $\pm$ 1.63d

Figures within parentheses are percentage over control.

The values with a common letter(s) do not differ significantly ( $P=0.05$ ).

Table 2. Adult mortality of *Syngizium cumini* fruit extracts at different concentrations recorded 24h after dipping (Mean  $\pm$  SE) (%)

Treatments	Adult mortality			
	0.5% Conc.	1.0% Conc.	2.0% Conc.	3.0% Conc.
Acetone	37.00 $\pm$ 2.51b (35.71)	49.00 $\pm$ 1.91b (47.95)	57.00 $\pm$ 2.51b (56.12)	66.00 $\pm$ 1.15b (65.30)
Ethanol	41.00 $\pm$ 1.91b (39.79)	52.00 $\pm$ 1.63b (51.02)	67.00 $\pm$ 1.91b (66.32)	72.00 $\pm$ 1.63b (71.42)
Methanol	51.00 $\pm$ 3.41a (50.00)	64.00 $\pm$ 2.82a (63.26)	79.00 $\pm$ 3.41a (78.57)	84.00 $\pm$ 1.63a (83.67)
Water	20.00 $\pm$ 1.63c (18.36)	32.00 $\pm$ 1.63c (30.61)	41.00 $\pm$ 1.91c (40.62)	53.00 $\pm$ 1.91c (52.04)
Control	2.00 $\pm$ 1.15d	2.00 $\pm$ 1.15d	2.00 $\pm$ 1.15d	2.00 $\pm$ 1.15d

Figures within parentheses are percentage over control.

The values with a common letter(s) do not differ significantly ( $P=0.05$ ).

Table 3. Ovicidal effect of *Syngizium cumini* fruit extracts at different concentrations recorded 7 days after exposure (Mean  $\pm$  SE) (%)

Treatments	Egg mortality			
	0.5% Conc.	1.0% Conc.	2.0% Conc.	3.0% Conc.
Acetone	56.00 $\pm$ 2.58b	63.00 $\pm$ 1.15b	66.00 $\pm$ 2.58b	74.00 $\pm$ 2.58b
Ethanol	71.00 $\pm$ 3.41a	76.00 $\pm$ 2.58a	88.00 $\pm$ 2.51a	95.00. $\pm$ 2.58a
Methanol	67.00 $\pm$ 2.58a	73.00 $\pm$ 1.91a	84.00 $\pm$ 1.91a	92.00 $\pm$ 1.63a
Water	20.00 $\pm$ 2.30c	31.00 $\pm$ 1.91c	43.00 $\pm$ 2.51c	57.00 $\pm$ 1.91c
Control	2.00 $\pm$ 1.15d	2.00 $\pm$ 1.15d	2.00 $\pm$ 1.15d	2.00 $\pm$ 1.15d

Values in the same column followed by different letters are significantly different at  $P < 0.05$  (Tukey's HSD test).

Table 4. Statistical comparison of LD<sub>50</sub> values of four different *S. cumini* fruit extracts against *T. urticae* adults and eggs

Treatments	Phase	LD <sub>50</sub> (%)	95% Confidence level		Regretion line	$\chi^2$ (df)
			Lower limit	Upper limit		
Acetone	Adult	0.375	0.127	1.099	Y= 4.682611 + 0.552773X	0.604(2)
	Egg	0.290	0.080	1.056	Y= 4.751284 + 0.536244X	0.554(2)
Ethanol	Adult	0.281	0.149	0.530	Y= 4.481631 + 1.152194X	3.038(2)
	Egg	0.233	0.120	0.454	Y= 4.526968 + 1.284322X	2.590(2)
Methanol	Adult	0.202	0.102	0.398	Y= 4.566181 + 1.420348X	1.041(2)
	Egg	0.255	0.129	0.506	Y= 4.533471 + 1.143614X	1.357(2)
Water	Adult	1.694	1.324	2.168	Y= 3.472274 + 1.24292X	0.088(2)
	Egg	2.516	1.870	3.385	Y= 3.141792 + 1.326591X	0.300(2)

- The original insect mortality data were corrected by Abbott's (1925) formula before analysis
- $\chi^2$  = Goodness of fit
- df= Degrees of freedom
- LD<sub>50</sub>= Median lethal dose
- The tabulated value of  $\chi^2$  is 5.99 ( df = 2)

Table 4 Repellency effects of *S. cumini* fruit extracts against *T. urticae* after 24h of exposure

Treatments	Concentration (%)	Repellency (%)	Repellency Class	No. of eggs/female after 24 h	
				Treated	Control
Acetone	0.5	34	II	2.43bc	39.87
	1.0	62	IV		
	2.0	78	IV		
	3.0	84	V		
Ethanol	0.5	42	III	1.93c	40.81
	1.0	64	IV		
	2.0	88	V		
	3.0	96	V		
Methanol	0.5	46	III	1.06c	22.06
	1.0	66	IV		
	2.0	84	V		
	3.0	94	V		
Water	0.5	4	I	4.87b	42.43
	1.0	16	I		
	2.0	38	II		
	3.0	58	III		
Control		32	II	32.50a	53.75

Means in the same column with a common letter are not significantly different at  $P < 0.05$  (Tukey's HSD test).



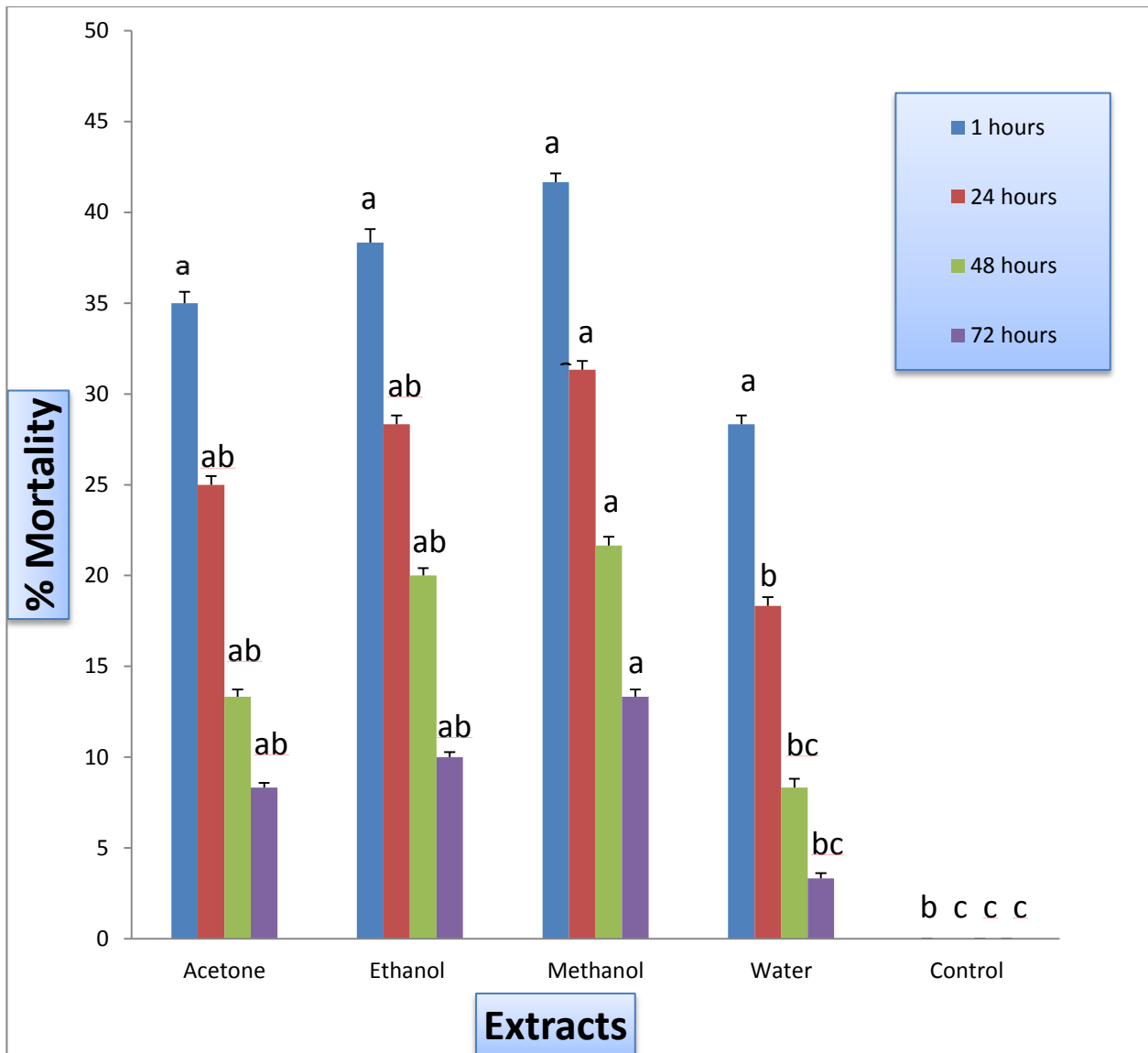


Figure 1. Persistence of *S. cumini* fruit extracts against *Tetranychus urticae* at 1, 24, 48 and 72 hours old LD<sub>50</sub> values. (Bar marked with same letter are not significantly different at  $P < 0.05$ ).

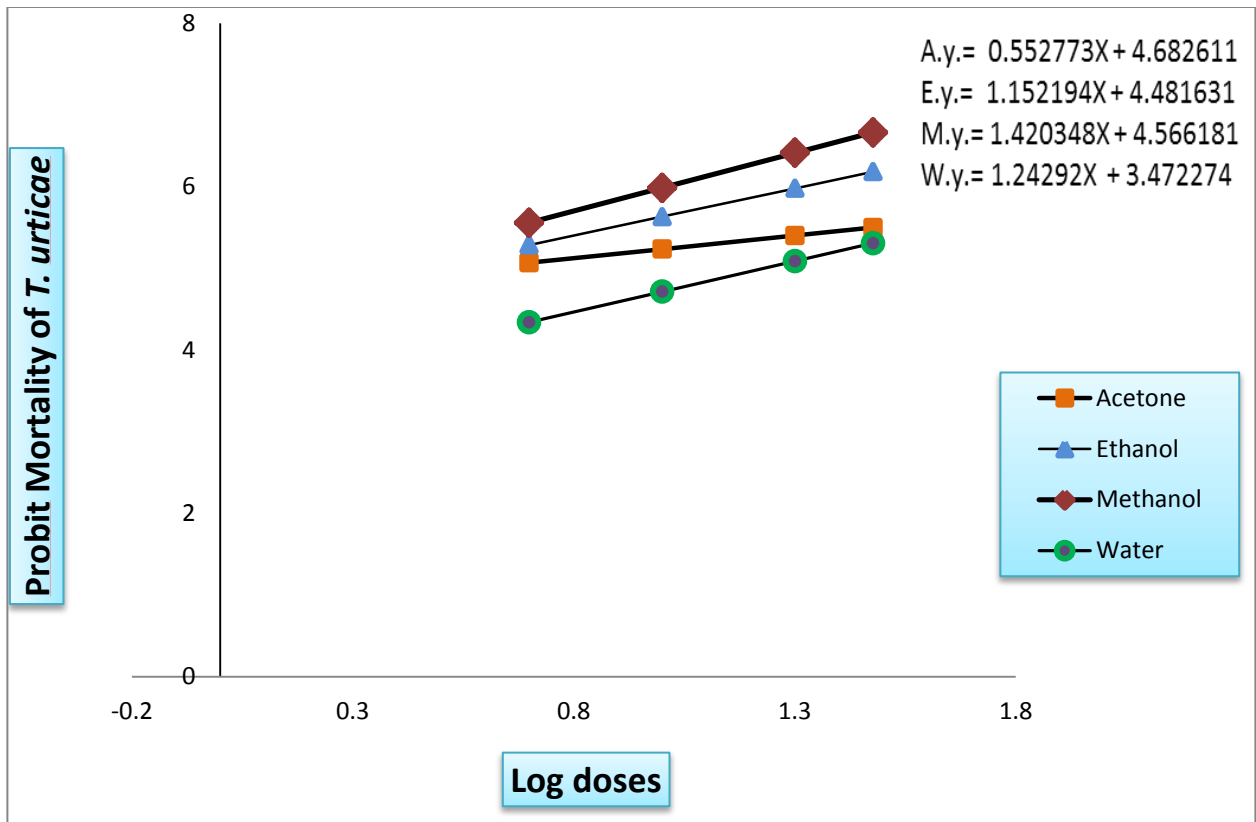


Figure 2. Relationship between probit mortality and log doses of *S. cumini* fruit extracts against *T. urticae*.

## CHAPTER V

### SUMMARY AND CONCLUSION

The present study was undertaken to investigate the effectiveness of *S. cumini* fruit extracts at different concentration for their toxicity and repellency effects against two-spotted spider mite, *T. urticae*. Mortality percentage of *T. urticae* adult females at 24 hour after treatment indicated that methanol extract possessed the highest mortality ( $97.00 \pm 1.00\%$ ;  $84.00 \pm 1.63\%$ ) in both leaf spraying and dipping method at 3.0% concentration whereas water extract showed the lowest mortality ( $28.00 \pm 1.63\%$ ,  $20.00 \pm 1.63\%$ .) at 0.5% concentration. Mortality percentage of *T. urticae* egg indicated that ethanol extract possessed the highest mortality ( $95.00 \pm 2.58\%$ ) at 3% concentration among all the fruit extracts whereas water extract showed the lowest mortality ( $20.00 \pm 2.30\%$ ) at 0.5% concentration. In probit analysis, the lowest  $LC_{50}$  value of methanol extract (0.202) indicated highest toxic effects and the highest  $LC_{50}$  values of water extract (1.324) indicated lowest toxic effects against adult females. In case of egg, the lowest  $LC_{50}$  value of methanol extract (0.233) indicated highest toxic effects and the highest  $LC_{50}$  values of water extract (2.616) indicated lowest toxic effects. The repellency class of different extracts at different concentration level varied between I to V. Ethanol extract was found more effective as repellent against *T. urticae* followed by methanol, acetone and water causing reduction in egg production per female by 96.73, 94.03, 92.50 and 85.00%, respectively. The extract also possessed residual effects on this pest. In the residual test, highest mortality (41.66%) in methanol extract at fresh (0 hour) treatment and water extract showed lowest mortality (3.33%) at 72 hour after treatment against *T. urticae*. Therefore methanol and ethanol extracts were found to be most effective to control the *T. urticae* in laboratory condition.

## REFERENCES

- Abbott W. S. 1925. A method of computing the effectiveness of insecticide. *Journal of Economic Entomology*. 18: 265-267.
- Abdullah A. A., El-Saiedy E. M. A., El-Fatih M. M. and Shoula M. E. 2012. Effect of some biological and biochemical control agents against certain squash pests. *Archives of Phytopatology and Plant Protection*. 45(1): 73-82.
- Adango E., Onzo A., Hanna R., Atachi P. and James J. 2006a. Inventaire de la faune des acariens sur *Amaranthus cruentus* (Amaranthaceae), *Solanum macrocarpon*, *Solanum aethiopicum* (Solanaceae) dans le Sud Benin. *International Journal of Tropical Insect Science*. 26: 155-165.
- Adango E., Onzo A., Hanna R., Atachi P. and James J. 2006b. Comparative demography of the spider mite, *Tetranychus ludeni* on two host plants in West Africa. *Journal of Insect Science*. 6: 1536-2442.
- Afify A. M. R., Ali F. S. and Turkey A. F. 2012. Control of *Tetranychus urticae* Koch by extracts of three essential oils of chamomile, marjoram and Eucalyptus. *Asian Pacific Journal of Tropical Biomedicine*. 2(1): 24-30.
- Afify A. M. R., El-Beltagi H. S., Fayed S. A. and Shalaby E. A. 2011. Acaricidal activity of different extracts from *Syzygium cumini* L. Skeels (Pomposia) against *Tetranychus urticae* Koch. *Asian Pacific Journal of Tropical Biomedicine*. pp. 359-364.
- Afify A. M. R., Fayed S. A., Shalaby E. A., and El-Shemy H. A. 2011. *Syzygium cumini* (pomposia) active principles exhibit potent anticancer and antioxidant activities. *African Journal of Pharmacy and Pharmacology*. 5(7): pp. 948-956.

- Afify A. M. R., El-Laithy A. Y . M., Shehata S. A. and El-Saiedy E. M. A. 2010. Resistance of strawberry plants against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). Trends in Acarology. pp. 505-507.
- Ahmadi M., Fathipour Y. and Kamali K. 2007. Population growth parameters of *Tetranychus urticae* (Acari: Tetranychidae) on different bean varieties. Journal of Entomological Society of Iran. 26(2): 1-10.
- Akyazi R., Soysal M. and Hassan E. 2015. Toxic and repellent effects of *Prunus laurocerasus* L. (Rosaceae) extracts against *Tetranychus urticae* Koch (Acari: Tetranychidae). Turkish Journal of Entomology. 39(4): 367-380.
- Antonious, G. F., Meyer J. E. and Snyder J. C. 2006. Toxicity and repellency of hot pepper extracts to spider mite, *Tetranychus urticae* Koch. Journal of Environmental Science and Health. 41: 1383-1391.
- Aslan, I., Ozbek H., Calmasur O., and Sahin F. 2004. Toxicity of essential oil vapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemesia tabaci* Genn. Industrial Crops and Produce. 19(2): 167-173.
- Atanassov N. 1997. Effect of the spider mite *Tetranychus urticae* Koch (Acarina: Tetranychidae) on cucumber yield. Biotechnology and Biotechnological Equipment. 11: A36-A37.
- Auamcharoen W. and Chandrapatya A. 2015. Potential Control of Two-Spotted Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) by Crude Extracts of *Duabanga grandiflora* (Lythraceae) and *Diospyros cauliflora* (Ebenaceae). Pakistan Journal of Zoology. 47(4): 953-964.
- Avery D. J. 1962. Book Review: The Church and the Gospel. <https://doi.org/10.1177/004056396202300218>

- Avery D. J. and Briggs J. B. 1968. The aetiology and development of damage in young fruit trees infested with fruit tree red spider mite, *Panonychus ulmi* (Koch). *Annals of Applied Biology*. 61(2): 277-288.
- Ayyanar M. and Subash-Babu P. 2012. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pacific Journal of Tropical Biomedicine* 2(3): 240-246. [http://dx.doi.org/10.1016/S2221-1691\(12\)60050-1](http://dx.doi.org/10.1016/S2221-1691(12)60050-1)
- Badway M. E. I., El-arami S. A. A. and Abdelgaleil S. A. M. 2010. Acaricidal and quantitative structure activity relationship of monoterpenes against the two-spotted spider mite, *Tetranychus urticae*. *Experimental and Applied Acarology*. 52: 261-274.
- Bajpai M. Pande A., Tewari S. K. and Prakash D. 2005. Phenolic constituents and antioxidant activity of some food and some medicinal plants. *International Journal of Food Science and Nutrition*. 56: 287-291.
- Baptiste S. J., Bloem k., Reitz R. and Mizell R. 2003. Use of radiation to sterilize two-spotted spider mite (Acari: Tetranychidae) eggs used as a food source for predatory mites. *Florida Entomologist*. 86(4): 389-394.
- Benelli G., Pavela R., Canale A., Nicoletti M., Petrelli R., Cappellacci L., Galassi R. and Maggi F. 2017. Isofuranodiene and germacrone from *Smyrniium olusatrum* essential oil as acaricides and oviposition inhibitors against *Tetranychus urticae*: impact of chemical stabilization of isofuranodiene by interaction with silver triflate. *Journal of Pest Science*. 90(2). 693-699.
- Bensoussan N., Santamaria M. E., Zhurov V., Diaz I., Grbić M. and Grbić V. 2016. Plant-herbivore interaction: dissection of the cellular pattern of *Tetranychus urticae* feeding on the host plant. *Frontiers in Plant Science*. 7: 1105.

- Bhandary M. J., Chandrashekar K. R. and Kaveriappa K. M. 1995. Medical ethnobotany of the siddis of Uttara Kannada district, Karnataka, India. *Journal of Ethnopharmacology*, 47: 149-158. [http://dx.doi.org/10.1016/0378-8741\(95\)01274-H](http://dx.doi.org/10.1016/0378-8741(95)01274-H)
- Biswas G. C., Islam W., Haque M. M., Saha R. K., Hoque K. M. P., Islam M. S. and Haque M. E. 2004. Some biological aspects of carmine mite, *Tetranychus cinnabarinus* Boisd, (Acari: Tetranychidae) infesting egg-plant from Rajshahi. *Journal of Bio-science*. 4(5): 588-591.
- Busvine J. R. 1971. A critical review of the techniques for testing insecticides. London.
- Calmasur O., Aslan I. and Sahin F. 2006. Insecticidal and acaricidal effect of three Lamiaceae plant essential oils against *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Industrial Crops and Products*. 23:140-146.
- Campbell R. J., Grayson R. L. and Marini R. P. 1990. Surface and ultrastructural feeding injury to strawberry leaves by the two spotted spider mite. *Horticultural Science*. 25: 948-951.
- Carbonnelle S. and Hance T. 2004. Cuticular lobes in the *Tetranychus urticae* complex (Acari: Tetranychidae): a reliable taxonomic character. *Belgian Journal of Zoology*. 134: 51-54.
- Chase M. W. and Reveal J. L. 2009. Phylogenetic classification of land plants to accompany APG III. *Botanical Journal of Linnean Society*. 161: 122-127.
- Chaudhuri W. M., Akbar S. and Rasool A. 1985. Studies on biosystematics and control of mites of field crops, vegetable and fruit plants in Pakistan, U. A. F., *Technical Bulletin*. 3: 314.
- Chaudhary B. and Mukhopadhyay K. 2012. *Syzygium cumini* (L.) Skeels: A potential source of nutraceuticals. *International Journal of Pharma and Bio Science*. 2: 2230-7605.
- Chaisson H. A., Bostanian N. and Vincent C. 2004. Acaricidal properties of a Chenopodium based botanical. *Journal of Economic Entomology*. 97: 1373-1377.

- Choi W., Lee S., Park H. and Ahn Y. 2004. Toxicity of plant essential oils to *Tetranychusurticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). Journal of Economic Entomology. 97(2): 553-558.
- Cichewicz R. H. and Kouzi S. A. 2004. Chemistry, biological activity, and chemotherapeutic potential of betulinic acid for the prevention and treatment of cancer and HIV infection. Medicinal Research Reviews. 24(1): 90-114.
- Clercq E. De. 2001. New developments in anti-HIV chemotherapy. Current Medicinal Chemistry. 8(13): 1543-1572.
- Collins R. D. and Margolies D. C. 1995. The effect of interspecific mating on sex ratios in the two-spotted spider mite and the banks grass (Acari: Tetranychidae). Journal of Insect Behavior. 8: 189-206.
- Dariusz G. 2003. Species composition of tetranychid mites (Acari: Tetranychidae) and predatory mites (Phytoseiidae) occurring on raspberry plantations in Poland. Journal of Plant Protection Research. 43: 353-360.
- Daulatabad C. M. J., Mirajkar A. M., Hosamani K. M. and Mulla M. M. G. 2006. Epoxy and cyclopropanoid fatty acids in *Syzygium cumini* seed oil. Journal of the Science of Food and Agriculture. 43(1): 91-94.
- Dupont L. M. 1979. On gene flow between *Tetranychus urticae* Koch. 1836 and *Tetranychus cinnabarinus* (Boisduval) Boudreaux, 1956 (Acari: Tetranychidae): synonymy between the two species. Entomologia Experimentalis et Applicata. 25: 297-303.
- Elizabeth E. G., Yuling O. and Rebecka A. S. 1997. Predaceous mites (Acari: Phytoseiidae) for control of spider mites (Acari: Tetranychidae) in nursery citrus. Journal of Environmental Entomology. 26: 121-130.



- El-Sharabasy H. M. 2010. Acaricidal activities of *Artemisia judaica* L. extracts against *Tetranychus urticae* Koch and its predator *Phytoseiulus persimilis* Athias Henriot (Tetranychidae : Phytoseiidae). *Journal of Biopesticides*. 3(2): 514-519.
- Endo S. and Tsurumachi M. 2001. Insecticide susceptibility of the brown plant hopper and the white back plant hopper collected from Southeast Asian *Journal of Pest Science*. 26 (1): 82-86.
- Erdogan P. and Yilmaz B. S. 2017. Acaricidal Activity of Extracts of *Juglans regia* L. on *Tetranychus urticae* Koch (Acari: Tetranychidae). *Journal of Food Science and Engineering*. 7: 202-208.
- Erdogan P., Yildirim A. and Sever B. 2012. Investigations on the Effects of Five Different Plant Extracts on the Two-Spotted Mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae). *Psyche: A Journal of Entomology*. Hindawi Publishing Corporation. p. 5. <https://doi.org/10.1155/2012/125284>
- Fasulo T. R. and Denmark H. A. 2000. Two-spotted spider mite, *Tetranychus urticae* Koch. UF/IFAS Featured Creatures EENY-150.
- Finney S. W. 1947. Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge University Press, London. pp. 333.
- Freitas A., Oliveira R., Nabity P., Higley L. and Fernandes O. 2009. Photosynthetic response of soybean to two-spotted spider mite (Acari Tetranychidae) injury. *Brazilian Archives of Biology and Technology*. 52: 825-834.
- Ganai S. A., Ahmad H., Sharma D., Sharma S., Khaliq N., Norboo T. and Chaand D. 2017. Management of red spider mite (*Tetranychus urticae* Koch.) infesting marigold (*Tagetes*

- erecta* L.) in jammu region. International Journal of Current Microbiology and Applied Sciences. 6(8): 168-174.
- Gapud V. P. 1981. Insect and mite pest of plant crops on Bangladesh and their natural enemies. In: A Compendium for biological control in IPM. United States Agency for International Development Agriculture Research Council/Chechiand Company Consulting Inc. p. 265.
- Gatarayiha M. C., Laing M. D. and Miller R. M. 2010. Combining applications of potassium silicate and *Beauveria bassiana* to four crops to control two-spotted spider mite, *Tetranychus urticae* Koch. International Journal of Pest Management. 56: 291-297.
- Geng S., Chen H., Zhang J. and Tu H. 2014. Bioactivity of Garlic-Straw Extracts Against the Spider Mites, *Tetranychus urticae* and *T. viennensis*. Journal of Agriculture and Urban Entomology. 30: 38-48.
- Ghaderi S., Kambiz M., Rowshan V. and Ghamadyari M. 2013. Toxicity and ovicidal activity of different plant extracts on two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Archives of Phytopathology and Plant Protection. 46 (1) : 120 - 126.
- Grbic, M., Van Leeuwen T. and Rodrigo M. 2011. The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. Nature. 479: 487-492.
- Greco N. M., Pereyra P. C. and Guillade A. 2006. Host-plant acceptance and performance of *Tetranychus urticae* (Acari: Tetranychidae). Journal of Applied Entomology. 130: 32-36.
- Hasanuzzaman M., Islam W. and Parween S. 2015. Repellent activities of *Syzygium cumini* L. (Myrtaceae) extracts against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). University Journal of Zoology, Rajshahi University. 34: 33-38.

- Huang L. Yuan X. Aiken C. and Chen C. H. 2004. Bifunctional anti-human immunodeficiency virus type 1 small molecules with two novel mechanisms of action. *Antimicrob Agents Chemother.* 48 (2): 663-665.
- Ibrahim C. and Tulin A. 2003. Investigation on phytophagous mites, their natural enemies and population fluctuations of important species on fig trees in Aydin (Turkey). *Turkish Journal of Entomology.* 27: 27-38.
- Ismail M. S., Soliman M. F., El Naggat M. H. and Ghallab M. M. 2007. Acaricidal activity of spinosad and abamectin against two-spotted spider mites. *Experimental Applied Acarology.* 43: 129-135. DOI: 10.1007/s10493-007-9108-8
- Jabeen K. & Javaid A. 2010. Antifungal activity of *Syzygium cumini* against *Ascochyta rabiei* the cause of chickpea blight. *Natural Product Research,* 24, 1158-1167. <http://dx.doi.org/10.1080/14786410902941154>
- Jeppson L. R., Keifer H. H. and Baker E. W. 1975. Mites injurious to economic plants. University of California Press, Berkeley. p. 614.
- Jimenez L. O. 2014. MS Thesis. Impact of early infestation of two-spotted spider mites (*Tetranychus urticae*) on cotton growth and yield. University of Arkansas, Fayetteville.
- Karin F. S. C., Jose O. G., De Lima and Giberto S. A. 2004. Predaceous mites in papaya (*Carica papaya* L.) orchards: in search of biological control agent of phytophagous mite pests. *Neotropical Entomology.* 33: 799-803.
- Kim D. I., Park J. D., Kim S. G., Kuk H., Jang M. S. and Kim S. S. 2005. Screening of some crude plant extracts for their acaricidal and insecticidal efficacies. *Journal of Asia-Pacific Entomology,* 8: 93-100.

- Koch C. L. 1836. Die Arachniden. Getreu nach der Natur abgebildet und beschrieben, C.H. Zeh and Johann Ludwig Lotzbeck, Nuremberg. 16: 1831-1848.
- Kumar S. V., Chinniah C., Muthiah C. and Sadasakthi A. 2010. Management of two-spotted spider mite *Tetranychus urticae* Koch, a serious pest of brinjal, by integrating bio rational methods of pest control. Journal of Biopesticides. 3: 361-368.
- Kumaran N. 2011. Within plant and within-leaf dispersion pattern of two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) on okra. Archives of Phytopatology and Plant Protection. 44: 1949-1957.
- Kumral N. A., Çobanoğlu S. and Yalcin C. 2009. Acaricidal, repellent and oviposition deterrent activities of *Datura stramonium* L. against adult *Tetranychus urticae* Koch. Journal of Pest Science. DOI 10.1007/s10340-009-0284-7
- Landeros J., Cerda P., Badii M. H., Aguirre L. A., Cerna E. and Ochoa Y. M. 2013. Functional response of *Neoseiulus californicus* on *Tetranychus urticae* on apple leaves. Southwestern Entomologist. 38: 79-84.
- Landeros J., Mora N., Badii M., Cerda P. A. and Flores A. E. 2002. Effect of sublethal concentrations of avermectin on population parameters of *Tetranychus urticae* on strawberry. Southwestern Entomologist. 27: 283-289.
- Liang G. M., Chen W., Liu T. X., 2003. Effects of three neem based insecticides on diamondback moth (Lepidoptera: Plutellidae). Crop Protection. 22: 333-40.
- Lienk S. E., Walve C. M. and Weives R. W. 1980. Phytophagous and predator mite on apple in New York. Search Agriculture (Geneva N Y). No. 6.
- Lindquist E. E. 1985. External anatomy. In spider mites, their biology, natural enemies and control. Vol. 1B (W. Helle and M. W. Sabelis eds.). Elsevier, Amsterdam. pp. 3-28.

- Maciel A. G. S., Rodrigues J. S., Trindade R. C. P., Silva E. S., Sant'Ana A. E. G. and Lemos E. P. 2015. Effect of *Annona muricata* L. (1753) (Annonaceae) seeds extracts on *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae). African Journal for Agricultural Research. 10(48): 4370-4375.
- Maria S., Carmen C., Juan M. A., Rafael M., Rafael L. and Jaun C. 2013. Genetic mapping of two QTL from the wild tomato *Solanum pimpinellifolium* L. controlling resistance against two spotted spider mite (*Tetranychus urticae* Koch). Theoretical and Applied Genetics. 126: 83-92.
- Matsuda T., Fukumoto C., Hinomoto N. and Gotoh T. 2013. DNA-based identification of spider mites: Molecular evidence for cryptic species of the genus *Tetranychus* (Acari: Tetranychidae). Journal of Economic Entomology. 106(1): 463-472.
- McDonald L. L., Guy R. H. and Speirs R. D. 1970. Preliminary evaluation of new candidate minerals as toxicants, repellents and attractants against stored product insects-I. Marketing Research Report No.882. Agricultural Research Service, U. S. A. Department of Agriculture. Washington D. C. p. 8.
- McGovern T.P., Gillenwater H. B. and McDonald L. L. 1977. Repellents for adults *Tribolium confusum*: mandelates. Journal of Georgia Entomological Society. 12: 79-89.
- Mersino E. 2002. Mites on ornamentals. Miscellaneous Pests. Cooperative Extension Services, College of Tropical Agriculture and Human Resources, Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa. pp. 1-3.
- Migeon, A. and Dorkeld, F. 2011. Spider mites web: a comprehensive database for the Tetranychidae. <http://www.montpellier.inra.fr/CBGP/spmweb>

- Miresmailli S. and Isman M. B. 2006. Efficacy and persistence of rosemary oil as an acaricide against twospotted spider mite (Acari: Tetranychidae) on greenhouse tomato. *Journal of Economic Entomology*. 99(6): 2015-2023.
- Mobley K. N. and Marini R. P. 1990. Gas exchange characteristics of apple and peach leaves infested by European red mite and two spotted spider mite. *Journal of the American Society for Horticultural Science*. 115: 757-761.
- Mothes U. and Seitz K. 1982. Action of the metabolic and chitin synthesis inhibitor Nikkomycin on the mite *Tetranychus urticae*; an electron microscope study. *Pesticide Science*. 13(4): 426-441.
- Moussa A. M., Emam A. M., Mohamed M. A. and Diab Y. M. 2010. In vitro evaluation of some Egyptian plants against the rot bacteria and spider mite and isolation the active constituent(s) from *Myrtus communis* leaves. *International Food Research Journal*, 17: 287-294.
- Nachman G., Zemek R. 2002. Interactions in a tritrophic acarine predator-prey metapopulation system III: Effects of *Tetranychus urticae* (Acari: Tetranychidae) on host plant condition. *Experimental & applied acarology*. 25: 27-42.
- Naher N., Islam W., Khalequzzaman M. and Haque M. M. 2008. Study on the developmental stages of spider mite (*Tetranychus urticae* Koch) infesting country bean. *Journal of Bioscience*. 16: 109-114.
- Negin G., Katayoon L., Mahmoud and Asghar A. T. 2013. Reproductive parameters and life expectancy of *Tetranychus urticae* on 12 genotype of melon and cucumber in laboratory condition. *Archives of Phytopatology and Plant Protection*. 46: 971-979.

- Numa S., Rodríguez L., Rodríguez D. and Coy-Barrera E. 2015. Susceptibility of *Tetranychus urticae* Koch to an ethanol extract of *Cnidocolus aconitifolius* leaves under laboratory conditions. Springer Plus. 4: 338.
- Pareek A., Meena R. K. and Yadav B. 2015. Antimicrobial activity of *Syzygium cumini*. Industrial Journal of Applied Research. 5(9): 64-66.
- Park Y. L. and Lee J. H. 2002. Leaf cell and tissue damage of cucumber caused by two-spotted spider mite (Acari: Tetranychidae). Journal of Economic Entomology. 95: 952-957.
- Pavela R. 2015a. Acaricidal properties of extracts and major furanochromenes from the seeds of *Ammi visnaga* Linn. against *Tetranychus urticae* Koch. Industrial Crops Products. 67: 108-113.
- Pavela R. 2016. Acaricidal Properties of Extracts of Some Medicinal and Culinary Plants against *Tetranychus urticae* Koch. Plant Protection Science. 52: 54-63.
- Pavela R., Dall'Acqua S., Sut S., Baldan V., Kamte S. L. N., Nya P. C. B., Cappellacci L., Petrelli R., Nicoletti M., Canale A., Maggi F. and Benelli G. 2016. Oviposition inhibitory activity of the Mexican sunflower *Tithonia diversifolia* (Asteraceae) polar extracts against the two-spotted spider mite *Tetranychus urticae* (Tetranychidae). Physiological and Molecular Plant Pathology 30: 1-8.
- Pavela, R. (2017) Extract from the roots of *Saponaria officinalis* as a potential acaricide against *Tetranychus urticae*. Journal of Pest Science. 90: 683-692. <https://doi.org/10.1007/s10340-016-0828-6>
- Ping-Man So. 1991. Distribution patterns of and sampling plans for *Tetranychus urticae* Koch (Acari: Tetranychidae) on roses. Researches on Population Ecology. 33(2): 229-243.
- Powell C. and Lindquist R. 1997. Spider mites (Acari-Tetranychidae). Ball Publishing, Batavia.

- Rabiea B., Manzar Z., Ahmad U., Shahnaz A. and Azam Z. 2011. Antihyperlipidaemic effects of *Eugenia jambolana* fruit in diet induced hyperlipidaemic rats. *Journal of Pakistan Medicine Association* 61(5): 433-437.
- Rafiullah M. R. M., Siddiqui A. W., Mir S. R., Ali M., Pillai K. K. and Singh S. 2006. Antidiabetic activity of some Indian medicinal plants. *Pharmaceutical Biology*. 44: 95-99.
- Raghavendra K. V., Chinniah C., Jayasimha G. T. and Gowthami R. 2017. Bio-efficacy of plant derivatives and natural oils against two spotted spider mite, *Tetranychus urticae* Koch. *Journal of Entomology and Zoology Studies*. 5(5): 1456-1461.
- Ravi K., Ramachandran B. and Subrmanian S. 2004. Protective effect of *Eugenia jambolana* seeds kernel on tissue P antioxidant in streptozotocin induced diabetic rats. *Biological and Pharmacological Bulletin*. 27: 1212-1217.
- Ravi K., Ramachandran B. and Subrmanian S. 2004. Protective effect of *Eugenia jambolana* seed kernel on antioxidant defence system in streptozotocin induced diabetic rats. *Life Science*. 75: 2717-2731.
- Raworth D. A., Gillespie D. R., Roy M. and Thistlewood H. M. A. 2002. *Tetranychus urticae* Koch, two-spotted spider mite (Acari: Tetranychidae). In Peter G. Mason and John Theodore Huber. *Biological Control Programmes in Canada*. CAB International. pp. 259-265.
- Reddall A., Sadras V. O., Wilson L. J. and Gregg P. C. 2004. Physiological responses of cotton to two-spotted spider mite damage. *Crop Science*. 44 (3): 835-846.



- Renata S. M., Denise N., Ivone R. D., Philippe A. and Navajas M. 2011. A critical review on some closely related species of *Tetranychus sensu stricto* (Acari: Tetranychidae) in the public DNA sequences databases. *Experimental and Applied Acarology*. 55: 1-23.
- Rincón R. A., Rodríguez D. and Coy-Barrera E. 2019. Botanicals Against *Tetranychus urticae* Koch Under Laboratory Conditions: A Survey of Alternatives for Controlling Pest Mites. *Plants*. 8: 272.
- Robertson J. L., Russell R. M., Saven N. E. and Preisler H. K. 2007. *Bioassays with Arthropods*. 1<sup>st</sup> Edition., C. R. C. Press, London. pp. 224.
- Ronaldo L., Amnon L., Shepard B. M., Alvin M. S. and Michael J. D. 2005. Sources of resistance to two-spotted spider mite (Acari: Tetranychidae) in *Citrullus* spp. *Horticultural Science*. 40: 1661-1663.
- Saber S. A. 2004. Influence of *Artemisia monosperma* Del. extracts on repellency, oviposition deterrence and biological aspects of the two-spotted spider mite *Tetranychus urticae* Koch. *Egyptian Journal of Pest Control*. 14: 34-348.
- Salman S. Y., Saritas S., Kara N. and Ay R. 2014. Acaricidal and Ovicidal Effects of Sage (*Salvia officinalis* L.) and Rosemary (*Rosmarinus officinalis* L.) (Lamiaceae) Extracts on *Tetranychus urticae* Koch (Acari: Tetranychidae). *Journal of Agricultural Science*. 20: 358-367.
- Sances F. V. Toscano N. O., La Pre L. F., Oatman E. R. and Johnson M. W. 1982. Spider mites can reduce strawberry yields. *California agriculture*. (1-2):14-16.
- Shafi P. M., Rosamma M. K., Jamil K. and Reddy P. S. 2002. Antibacterial activity of *Syzygium cumini* and *Syzygium tra\_ancoricum* leaf essential oils - Short report. *Fitoterapia*, 73 (5): 414-416. [http://dx.doi.org/10.1016/S0367-326X\(02\)00131-4](http://dx.doi.org/10.1016/S0367-326X(02)00131-4)

- Sharma A. and Pati P. K. 2012. First record of the carmine spider mite, *Tetranychus urticae* infesting *Withania somnifera* in India. *Journal of Insect Science*. 12(50): 1-4.
- Si-Jun Z., Jeroen van Dijk P., Maaike B. and Marcel D. 2007. Sensitivity and speed of induced defense of cabbage (*Brassica oleracea* L.): Dynamics of BoLOX expression patterns during insect and pathogen attack. *Molecular Plant-Microbe Interactions*. 20: 1332-1345.
- Sing R. N. and Saratchandra B. 2005. The development of botanical products with special reference to Seri-ecosystem. *Caspian Journal of Environmental Science*. 3: 1-8.
- Su H. H., Jiang F., Yu M. Z., Yang X. M., Yang Y. Z. and Hong X. Y. 2012. Effect of *Wolbachia* on rDNA-ITS2 variation and evolution in natural populations of *Tetranychus urticae* Koch. *Annals of the Entomological Society of America*. 78: 609-614.
- Takafuji A. and Kamibayashi M. 1984. Life cycle of a non-diapausing population of the two-spotted spider mite, *Tetranychus urticae* Koch in a pear orchard. *Researches on Population Ecology*. 26: 113-123.
- Takafuji A., Ozawa A., Nemoto H. and Gotoh T. 2000. Spider mites of Japan: their biology and control. *Experimental and Applied Acarology*. 24(5-6): 319-335.
- Tomczyk A. and Kropczynska D. 1985. Effects on the host plant. In W. Helle and M. W. Sabelis (eds). *Spider Mites, Their Biology. Natural Enemies and Control*. Elsevier, Amsterdam. Vol 1A. pp. 312-330.
- Uesugi R., Goka K. and Osakabe M. 2002. Genetic basis of resistances to chlorfenapyr and etoxazole in the two-spotted spider mite (Acari: Tetranychidae). *Journal of Economic Entomology*. 95(6): 1267-1274.
- Van de Vrie M., McMurtry J. A. and Huffaker C. B. 1972. Biology, ecology and pest status, and host-plant relationships of Tetranychids. *Hilgardia*. 41(13): 343-432.

- Van Leeuwen T., Van Pottelberge S. and Tirry L. 2010. Acaricide resistance mechanisms in the two spotted spider mite *Tetranychusurticae* and other important Acari: a review. *Insect Biochemistry and Molicular Biology*. 40: 563-572.
- Vidal J., Carbajal A., Sisniegas M. and Bobadilla M. 2009. Toxic activity of *Argemone subfusiformis* Ownb. and *Tagetes patula* Link against *Aedes aegypti* L. fourth instar larvae and pupae. *Revista Peruana de Biologia* 15: 103–109.
- Wang Y. N., Shi G. L., Zhao L. L., Liu S. Q., Clarke S. R. and Sun J. H. 2007. Acaricidal activity of *Juglan sregiale*leaf extracts on a *tetranychus viennensis* and *Tetranychus cinnabarinus* (Acari: Tetranychidae). *Journal of Economic Entomology*. 100: 1298-1303.
- Weintraub P. and Palevsky E. 2008. Evaluation of the predatory mite, *Neoseiulus californicus* for spider mite control on greenhouse sweet pepper under hot arid field conditions. *Experimental and Applied Acarology*. 45: 29-37.
- Williamson E. M. 2002. Major Herbs of Ayurveda. Churchill Livingstone,China. 279-282.
- Yanar D., Kadioğlu I. and Gökçe A. 2011. Ovicidal activity of different plant extracts on two-spotted spider mite (*Tetranychus urticae* Koch) (Acari: Tetranychidae). *Scientific Research and Essays*. 6(14): 3041-3044.
- Yogeswari P. and Sriram D. 2005. Betulinic acid and its derivatives: a review on their biological properties. *Current Medicinal Chemistry*. 12: 323-349.
- Ziegler H. L., Franzyk H., Sairafianpour M., Tabatabai M., Tehrani M. D., Bagherzadeh K., Hagerstrand H., Stærka D. and Jaroszewskia W. J. 2004. Erythrocyte membrane modifying agents and the inhibition of Plasmodium falciparum growth: structure–activity relationships for betulinic acid analogues. *Bioorganic & Medicinal Chemistry*. 12: 119-127.