ACARICIDAL ACTIVITIES OF *ALOE VERA* L. LEAF EXTRACTS AGAINST *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE)

A THESIS

BY

RUMANA AKHTER BASUNIA

Registration No. 1605364

Session: 2016-2017

Semester: July-December, 2017

MASTER OF SCIENCE (MS)

IN

ENTOMOLOGY

DEPARTMENT OF ENTOMOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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Submitted to the Department of Entomology
Hajee Mohammad Danesh Science and Technology University, Dinajpur
in partial fulfillment of the requirements for the degree of

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

DECEMBER, 2017

Dedicated
to
My Beloved Parents

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The Authoress

Acaricidal activities of *Aloe vera* L. leaf extracts against *Tetranychus urticae* Koch (Acari: Tetranychidae)

ABSTRACT

Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is a very serious pest worldwide, causing considerable damage to vegetables, flower and fruit crops. The acaricidal, ovicidal and repellent activity of *Aloe vera* L. (Asphodelaceae) leaf extracts using four solvents were evaluated against *T. urticae* under laboratory conditions. The *A. vera* leaf extracts were tested against *T. urticae* at 0.25, 0.50, 1.0 and 2.0% concentrations. The results showed that all the extracts had toxic and repellent effects on two-spotted spider mite. Mortality percentage was gradually increased with the increase of doses. The acetone extract showed the highest mortality (95%) of adult females followed by ethanol (83%) at 2% concentration. The LC_{50} values of acetone, ethanol, methanol and petroleum ether extract for adult females were 0.446, 0.667, 0.953 and 1.279, respectively and for eggs were 0.950, 1.406, 2.115 and 3.312, respectively. Ethanol extract was found more effective as repellent against adult females of *T. urticae* followed by acetone, methanol and petroleum ether causing reduction in egg production per female by 96, 94, 85 and 83%, respectively. In residual test, the acetone extract showed the highest mortality (49%) at 1 hour after treatment followed by ethanol (43%), methanol (29%) and petroleum ether (25%). The percent of mortality decreased after 48 and 72 hours after treatments. The results suggested that *A. vera* has a great potentiality to develop as a botanical acaricide for *T. urticae* control.

Key words: *Aloe vera*, *Tetranychus urticae*, acaricidal activity, ovicidal activity, repellency

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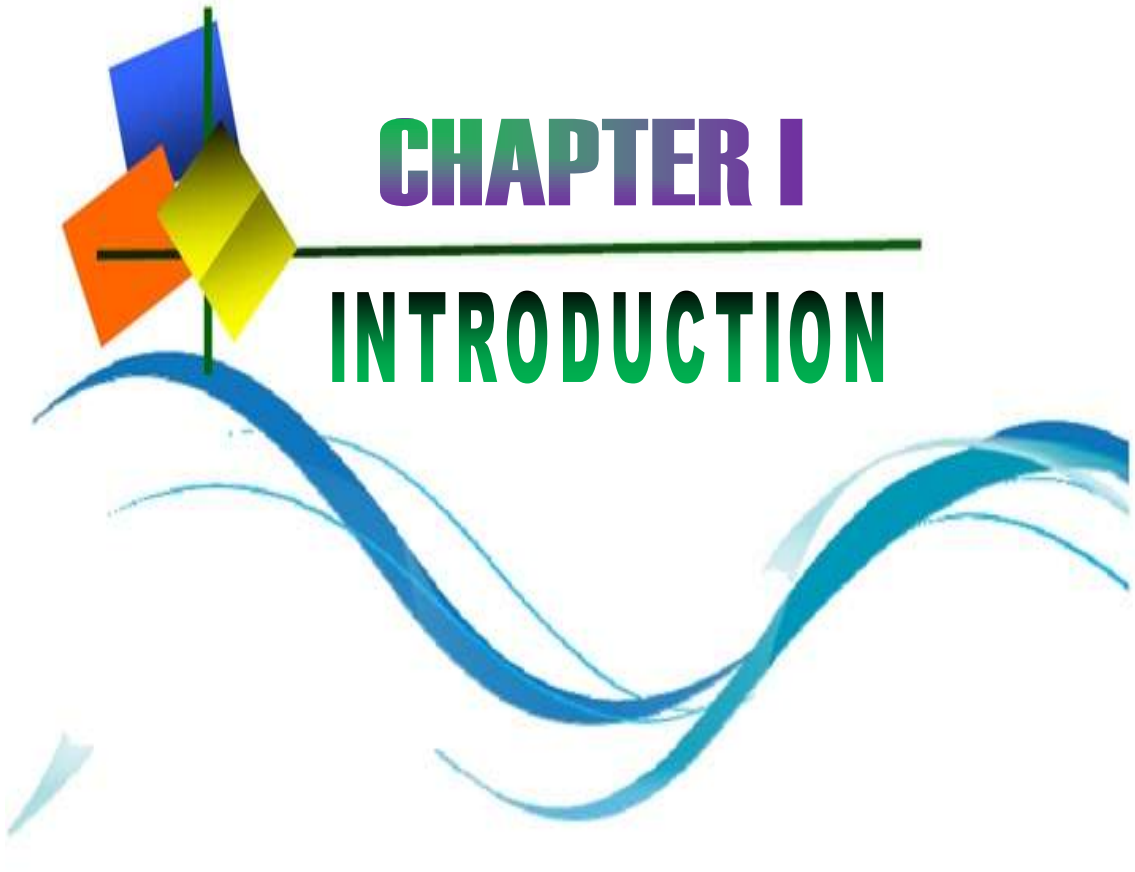

CHAPTER I

INTRODUCTION

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INTRODUCTION

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a cosmopolitan pest of greenhouse and field crops worldwide. It attacks a wide range of garden plants including ornamentals, fruit crops and vegetables and feeds on more than 1100 species of plants under 140 families (Vassiliou and Kitsis, 2013). The developmental rate of survival, reproduction and longevity of *T. urtcae* may vary in response to changes in temperatures, host plant species, host plant nutrition, cultivar type, phonology stage, exposure to pesticides, relative humidity etc. (Sabelis, 1981; Brandenburg and Kennedy, 1987b, Wermelinger *et al*., 1991; Dicke, 2000; Macic, 2003; Skorupska, 2004).

Adult and immature stages of *T. urticae* suck fluid from the lower surface of leaves (Park and Lee, 2002). Leaf yellowing, bronzing, defoliation and even plant death occur due to the direct effects of mite feeding (Helle and Sabelis, 1985; Mersino, 2002). Indirect effects of feeding may include a decrease in transpiration and photosynthesis which results in reduced yield (Brandenburg and Kennedy, 1987; Mathews and Tunstall, 1994).

For the past several decades, the control of *T. urticae* has depended mainly on applications of pesticides. The extensive use of pesticides has led to rapid development of resistance in this mite (Van Leeuwen *et al*., 2010). More than 80 pesticides belonging to different chemical groups have been reported on the development of resistance by *T. urticae* (Vassiliou and Kitsis, 2013). To achieve sustainable management of two-spotted spider mite (TSSM), it is therefore important to diminish the use of conventional acaricides or pesticides and to alternate them with products having a different mode of action (Choi *et al*., 2003, Lee *et al*., 2003). Numerous plant extracts have been reported to have a variety of biological activities against insects and mites including repellence,

feeding and oviposition deterrence, toxicity, and growth regulatory activity (Sing and Saratchandra, 2005). Moreover, plant based pesticides often contain a mixture of active substances, which can delay or prevent resistance development (Wang *et al*., 2007).

Aloe vera L., is a member of Asphodelaceae family, is a perennial, evergreen, herbaceous plant. Also known as medicinal aloe, the medicinal value of *A. vera* is widely recognized by the medical profession and traditional Chinese medicine practitioners. Aloe plants contain more than 160 chemical components and 80 or more used as medicinal ingredients (Deng, 2000; Fu *et al*., 2008; Yao *et al*., 2007). One of the compounds, aloin causes abnormal outer structures and configuration in bacterial cell walls (Warmer *et al*., 2003). *Aloe vera* also has good antibacterial, acaricidal, and insecticidal activities; it plays an important role in killing pests in granaries (Morsy *et al*., 2000). However, only its ovicidal activity and contact toxicity have been confirmed (Wei *et al*., 2011). *Aloe vera'*s biological activity is widely accepted and it is used for various medical, cosmetic and nutraceutical purposes. It also exhibits anti-oxidant, anti-viral, anti-bacterial, anti-fungal and anti-inflammation activities (Yan *et al*., 2001; Tian, 2002; Ni *et al.*, 2004; OuYang *et al*., 2008; Ndhlala *et al*., 2009; Wang, 2009) and it is used as a laxative, as protection against radiation, and in immune stimulation. Many aloe based products, ranging from drinks, gels, powders, capsules, creams, soaps and face tissues are available on the commercial market for human use and consumption. Compared to the benefits of aloe to humans, relatively little is known about its insecticidal and/or acaricidal activities. So, the objective of this study was to evaluate the acaricidal activity of *A. vera* L. extracts against *T. urticae.*

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Tetranychus urticae is one of the most common and serious mite pests worldwide which mainly infest crops and fruits. It is the widely known member of the family Tetranychidae. It is extremely small, barely visible with the naked eye as reddish or greenish spots on leaves and stems; the adult females measure about 0.4 mm long. It can be seen in greenhouses and tropical and temperate zones. *T. urticae* can increase population density very rapidly, produce numerous generations per year. Outbreaks of *T. urticae* in agricultural ecosystems result in large economic loss and cause serious ecological problems. In recent years, there is an increasing interest for natural pesticides which are derives from plants (Isman, 2000; Isman *et al*., 2006) because they are generally perceived to be safer than the synthetics. Plant extracts are one of several non-chemical control options that have recently received attention.

2.1 Taxonomy of *T. urticae*

Kingdom: Animalia

Phylum: Arthropoda

Sub- phylum: Chelicerata

Class: Arachnida

Sub-class: Acari

Super-order: Acariformes

Order: Prostigmata

Family: Tetranychidae

Genus: *Tetranychus*

Species: *Tetranychus urticae* (Koch, 1836)

2.2 Distribution of *T. urticae*

The two-spotted spider mite originates from temperate climates (Fasulo and Denmark, 2000). It was originally native only to Eurasia, but has acquired a cosmopolitan distribution (Raworth *et al.*, 2002). It was originally described from European specimens and considered to be a temperate zone species and distributed throughout the tropical and subtropical parts of the world (Jeppson *et al.*, 1975). *Tetranychus urticae* naturally occurred in following countries:

A. Asia

Bangladesh (Naher *et al*., 2008), Iran (Ghaderi *et al*., 2013), Japan (Matsuda *et al*., 2013), China (NE) (Su *et al*., 2012), China (NW) (Liu *et al*., 2006), China (South) (Su *et al*., 2012), India (Sharma and Pati, 2012), Turkey (Demirozer *et al*., 2011), Korea-South (Kawashima *et al*., 2008), Vietnam (Hinomoto *et al*., 2007) etc.

B. Australia

(South+SE) (Karimzadeh *et al*., 2004), New Zealand (Bowie *et al*., 2001) etc.

C. Europe

United Kingdom (Fitzgerald *et al*., 2008), Italy (Tirello *et al*., 2012), Germany (Weihrauch, 2005), Poland (Zaneta, 2012), Netherlands (Khajehali *et al*., 2011), Belgium (Pottelberge *et al*. 2009), France (Carbonnelle *et al*. 2007), Russia (Popov, 2003), Czech Republic (Vostrel, 2010), Spain (continental) (Ernestina *et al*., 2012). Serbia (Petrovic *et al*., 2010), Norway (Ingeborg *et al*., 2008), Lithuania (Uselis *et al*., 2006) etc.

D. America

Canada (Hardman *et al*., 2007; Raworth, 2007), Coastarica (Carl and Jose, 2005), Mexico (Badii *et al*., 2004; Landeros *et al*., 2004), U.S.A. (NE) (Gupta and Krischik, 2007), U.S.A. (NW) (Woods *et al*., 2012), Argentina (Greco *et al*., 2005), Brazil (NE) (Renata *et al*., 2011), Brazil (South) (Bernardi *et al*., 2013), Colombia (Braun *et al*., 1987), Guadeloupe (Carlos and Etienne, 2006).

E. Africa

South Africa (Gatarayiha *et al*., 2011), Egypt (Abdallah *et al*., 2012), Spain (Renata *et al*., 2011), Senegal (George, 2006), Kenya (Markus *et al*., 2006; Anyango, 2003), Tunisia (Navajas *et al*., 2000) etc.

2.3 Host range and specificity of *T. urticae*

The two-spotted spider mite, *T. urticae* is an extremely polyphagous pest that has been reported from more than 900 host species and 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 (Takafuji *et al*., 2000; 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). Its numerable hosts include field crops, ornamental and house plants, vegetables, forage crops and fruit trees. Among the tree fruits, apple, pear, peach, nectarine, apricot, cherry (sweet and sour), plum, and prune are suitable hosts. This mite causes considerable damage to eggplant, bean, melon, tomato, strawberry, pumpkin and many other outdoor and greenhouse crops (Chaudhri *et al*., 1985; Ahmadi *et al*., 2007) over the whole year. The host plants for *T. urticae* are as follows:

Vegetables: Cabbage (Si-Jun *et al*., 2007), Cucumber (Negin *et al*., 2013), Eggplant (Kumar *et al*., 2010), Green pepper/chilli (Weintraub and Palevsky, 2008), Okra and relatives (Kumaran, 2011), Onion/garlic/leek (Greco *et al*., 2006), Solanum (Adango *et al*., 2006a), Squash/pumpkin (Abdallah, 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 (Hidenari, 2002), Raspberry (Dariusz, 2003), 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).

A number of vegetable crops and ornamentals plant 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 Nature of damage of *T. urticae*

Adult and immature stages of *T. urticae* suck fluid from the lower surface of leaves (Park and Lee, 2002). Leaf yellowing, bronzing, defoliation and even plant death occur due to the direct effects of mite feeding (Helle and Sabelis, 1985; Mersino, 2002). Indirect effects of feeding may include a decrease in transpiration and photosynthesis which results in reduced yield (Brandenburg and Kennedy, 1987; Mathews and Tunstall, 1994).

2.5 Plant extracts against *T. urticae*

Sinchaisri *et al*. (1991) who evaluated 49 ethanol crude extracts from 44 plant species were tested for the insecticidal activity on third instar diamond back moth larvae under laboratory conditions. Three plant extracts (20mg/ml conc.) showed high insecticidal

activity (100% mortality of larvae); *Annoamuricata* seeds, *Annona squamosal* seeds and *Stemona collinsae* roots.

Safaa *et al*. (2015) conducted experiments to evaluate the toxicity effect against *Tetranychus urticae*. Result cleared that red pepper extract exhibited a high degree of efficiency against adult female of *T. urticae* recording overall mean of mortality ranged from 24.46-98.56% while the lowest was thyme recorded overall mean of mortality ranged from 0.00-66.86% for 1-6% concentration, respectively.

Kumral *et al.* (2010) showed that leaf and seed extracts from *Datura stramonium* had demonstrated acaricidal, repellent, and oviposition deferent activities against *T. urticae*. Whereas extracts of *Capparis aegyptia* (Capparaaceae) was toxic to *T. urticae* females.

Kundo *et al*. (2007) who investigated the repellent effect of biskatali plant extract in two solvent namely ethyl alcohol and chloroform against red flour beetle. They observed plant extract in chloroform solvent showed the highest repellency to *Tribolium castaneum* than in ethyl alcohol solvent.

Talukder and Howse (1995a and b) who reported that ethanol seed extracts of *Aphanamixis polystachya* had strong repellent effects on red flour beetle and rice weevil.

Monsur *et al*. (2004) found that twenty nine plant extracts of local species were evaluated for their potential as a source of bio active ingredients with significant acaricidal activity that could lead to the development of new and safe bioacaricides. The crude extracts of 70% ethanol of these plants were tested for their acaricidal activity against the carmine spider mite *T. cinnabarinus* in a bioassay under controlled conditions, and number of egg laid were measured. Extracts from *Eucalyptus camaldulensis* and *Inula viscosa* caused more than 25% mortality. Sixteen plant extracts

showed significant repellency and found to cause a significant reduction in the number of eggs laid compared with control.

Zhang *et al*. (2013) the aloe acetone extract against female carmine spider mite, the LC_{50} values to *T. cinnabarinus* were found to be 0.836 and 0.167 mg/ml for 42h and 72h respectively.

Wei *et al*. (2011) reported that *A. vera* leaf extracts of four different solvents against female adults of *T. cinnabarinus* revealed that all 4 extracts namely acetone, ethanol, ethyl acetate and water had considerable acaricidal activity. The three organic solvent extracts required 48h to achieve greater than 50% mortalities and water extract needed 72h. The acetone extracts show highest acaricidal activity than others.

Chermenskaya *et al*. (2010) that the ethanol extract of leaves of *Ailanthus altissima* L. (Simarubaceae) and roots of *Convolvulus krauseanus* Regel and Schmalh (Convolvulaceae) showed high acaricidal activity to *T. urticae*. In addition the extracts reduced the population of the next generation of *T. urticae.*

Auamcharoen *et al*. (2015) found that crude methanol extract of *Duabanga grandiflora* was found to have less toxicity with maximum mortality of 35.6% when compared to control while chloroform crude extract of *Diospyros cauliflora* showed 45.6% repellency which is equaled to those of *D. grandiflora* crude methanol extract.

Maciel *et al*. (2015) reported that the ethanolic extract of *Annona muricata* showed a good potential for the control of *T. urticae*, inducing mite mortality, egg toxicity, as well as repellency and residual effects.

Ahmed and Beg (2001) reported that extracts of ginger rhizomes, garlic bulbs and aloe vera were successfully used in controlling fungal pathogens.

Al-Alawi (2013) found that out of 18 plant extracts screened for acaricidal activity against *T. urticae*, the extracts of *Ruta chalpensis* (65%) and *Astragalus oocephalus* (55%) showed the highest activity. These findings open avenues for more research in isolation and identification of the active secondary metabolites that might be responsible for that activity in an attempt to develop botanical acaricides for the management of *T. urticae.*

Amer *et al*. (1989) revealed that diethyl ether extract of *Abrus precatorius* L. seeds had a high acaricidal activity against eggs of *T. urticae*, while water ethyl alcohol and acetone extracts were the most potent extracts against adult females of *T. urticae*.

Monsur *et al.* (1987) demonstrated that ethanol extract of neem (*Azaderachta indica*, *A. Juss*) seed kernel was the most potent extract (LC_{50}= 0.6 %) against the pest *T. cinnabarinus* (Boisd.).

Wang *et al*. (2007) revealed that the crude extract of walnut leaf had some contact and systemic effect on *T. cinnabarinus* and *T. viennensis*.

Barakat *et al.* (1984, 1985) found that some plant extracts such as devil's apple and lupin were the most potent extracts tested against *T. urticae*.

Tewary *et al*. (2005) who reported that the petroleum ether extract of *Hedera nepalensis* L. (Araliaceae) leaves induced 35 and 38% mortalities of *T. urticae* at 0.5 and 1%, respectively, 48 h after exposure. Moreover, the petroleum ether extract of *H. nepalensis* fruits revealed 36% mortality of *T. urticae* at 1%. The aqueous methanol extract of *H. nepalensis* fruits exhibited 32 and 65% mortalities of *T. urticae* at 0.5 and 1%, respectively. The petroleum ether and aqueous methanol extracts of root of *Berberis lycium* L. (Berberidaceae) demonstrated 26 and 43% mortalities at 1%. 48h after exposure.

Soliman *et al*. (2005) found that successive extracts of Artemisia with petroleum ether, chloroform, ethyl acetate and ethanol were toxic for the two-spotted spider mite *T. urticae*,

Ibrahim *et al*. (1993) reported that the flower ethanolic extract of the essential oil *Cotula cinerea* L. was the most toxic on eggs of *T. urticae*.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present study was conducted in the Entomology Laboratory of Agriculture Faculty, Hajee Mohammad Danesh Science and Technology University, (HSTU), Dinajpur, Bangladesh. The experimental periods were October 2017 to June 2018. All experiments were carried out under laboratory condition in an ambient temperature.

3.1 Mite rearing

The adult mites, *Tetranychus urticae* were collected from an infested bean field of Hajee Mohammad Danesh Science & Technology University campus in 2017. Fifty percent of the collected mites were introduced on the country bean leaves grown in plastic pot (20 D × 20 H cm). Another fifty percent of the mites were reared on bean leaves in Petri dishes (9 D × 2 H cm). Fresh bean leaves were provided to replace the old dried leaves in the Petri dishes.

Plate 1. Bean plant in pot

Plate 2. Rearing mites on leaves in Petri dish

3.2 Plant material collection

Mature fresh *Aloe vera* L. (Asphodelaceae: Asparagales) leaves were collected from Anando nursery, Paharpur, Dinajpur, Bangladesh.

Plate 3. *Aloe vera* Leaves

3.3 Chemical Reagent

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

3.4 Preparation of plant extracts

Aloe vera leaves were dried in shade. After that the leaves were cut into small pieces and finally again dried in an oven at 50˚C. The dried plant materials were grinded to make fine powders of 80 meshes for extraction with four different solvents (acetone, ethanol, methanol and petroleum ether). 100 grams of *A. vera* powder were taken in a 600 ml beaker and added 300 ml of acetone, ethanol, methanol and petroleum ether. Then the mixtures were stirred for 30 minutes by a magnetic stirrer (600 rpm) and shaked by hand and allowed to stand for 72 hours. Then, the mixtures were filtered through a filter paper

(Whatman No. 1). The filtered materials were taken into a volumetric flask and were allowed to evaporate the solvents with the help of rotary evaporator (50-60^0 C). Finally, crude extracts were preserved in tightly corked glass vials and stored in a refrigerator at 3^{o}C for experimental use.

A. Powders of *Aloe vera* leaves

B. Mixing of leaf powder with four solvents separately in conical flasks

C. Solutions were filtered in separate volumetric flasks

D. Preserved in a tightly corked glass vials

Plate 4. Techniques of different plant extracts preparation

3.5 Acaricidal effect on adult

Three centimeter diameter leaf discs were cut from the center of bean leaves. The leaf discs were placed on water saturated cotton in the Petri dish (9 D × 2 H cm) with the underside of the leaf facing upward. Twenty adult females (2days old) were transferred to each disc (4 leaf discs, 20 adult /disc). Leaf discs were sprayed with four different solvent extracts of *A. vera* at four different concentrations (0.25, 0.50, 1.0, and 2.0%). Controls were performed using distilled water in the experiment. 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 study to observe the alive and dead mite. Mortality percentage was calculated by using Abbott's corrected formula (Abbott, 1925).

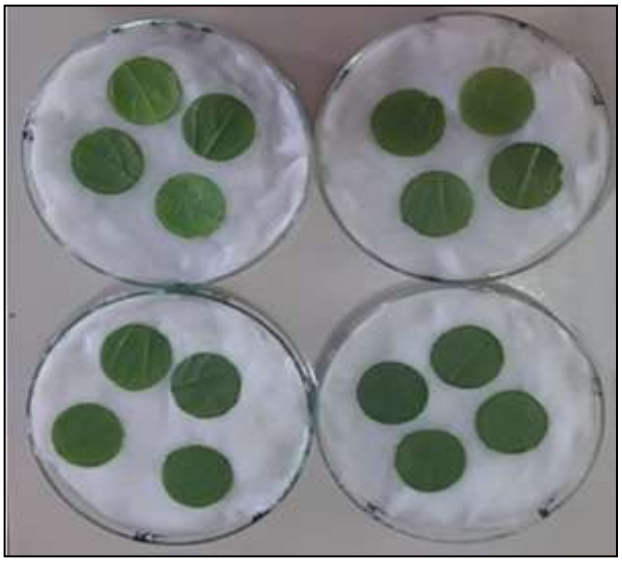


Plate 5. Treatments of adult females mite placed on leaf discs

3.6 Ovicidal effect

Bean leaf discs of approximately three centimeter in diameter were used as substrate to oviposition. Four leaf discs were used for each treatment and ten female mites were placed upside down on hydrophilic cotton in a Petri dish (9 D × 2 H cm) with water and allowed to 4 hours to lay eggs. After then, the adults were removed and the eggs were checked under stereomicroscope to ensure that at least 25 eggs on each leaf disc. The discs were treated with four different concentrations (0.25, 0.50, 1.0and 2.0%) of four different solvent extracts of *A. vera* with the help of hand sprayer. The numbers of hatched and unhatched eggs were recorded after seven days. A stereomicroscope (BST 606, Made in Germany) was used to record the number of hatched and unhatched eggs.

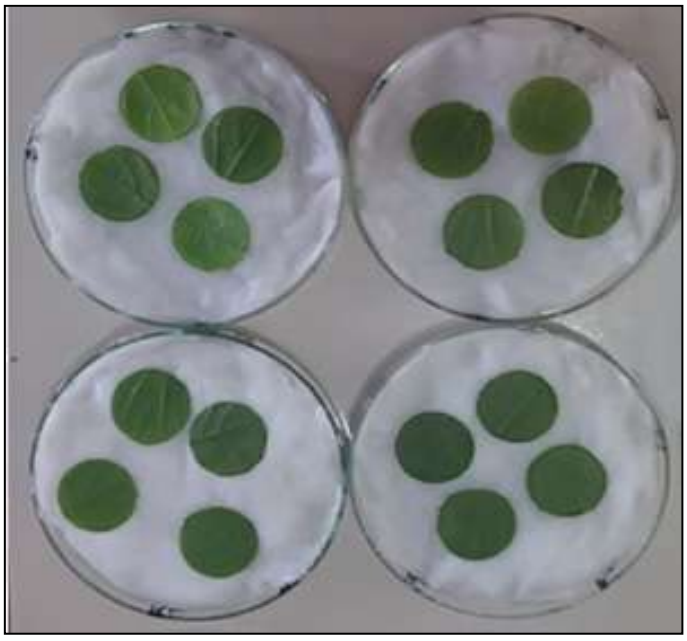


Plate 6. Treatments of eggs

Plate 7. Stereomicroscope (BST 606, Made in Germany)

3.7 Repellency effect

Leaf disc of bean along with midrib of 3 cm in diameter was used to evaluate the repellency of the four different solvent extracts of *A. vera* mixture. A half portion of the disc was dipped in the test concentrations of 10 seconds and the other half was used as a control. The treated discs were allowed to air dry for 30 minutes and then placed on moistened cotton in Petri dishes. Twenty adult females were put on the midrib of each disc. Experiment was replicated four times. The number of adult mites present on each half of the disc was recorded 24 hours after mite transfer. The number of eggs laid on both sides was also recorded under a stereo microscope. The data were expressed as percentage repellency (PR) which was computed using the following formula described by McDonald *et al.* (1970) with some modifications.

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

Where, N_C is the percentage of mites present in the untreated half of the disc. Positive (+) values expressed repellency and negative (-) values attractancy. The mean values

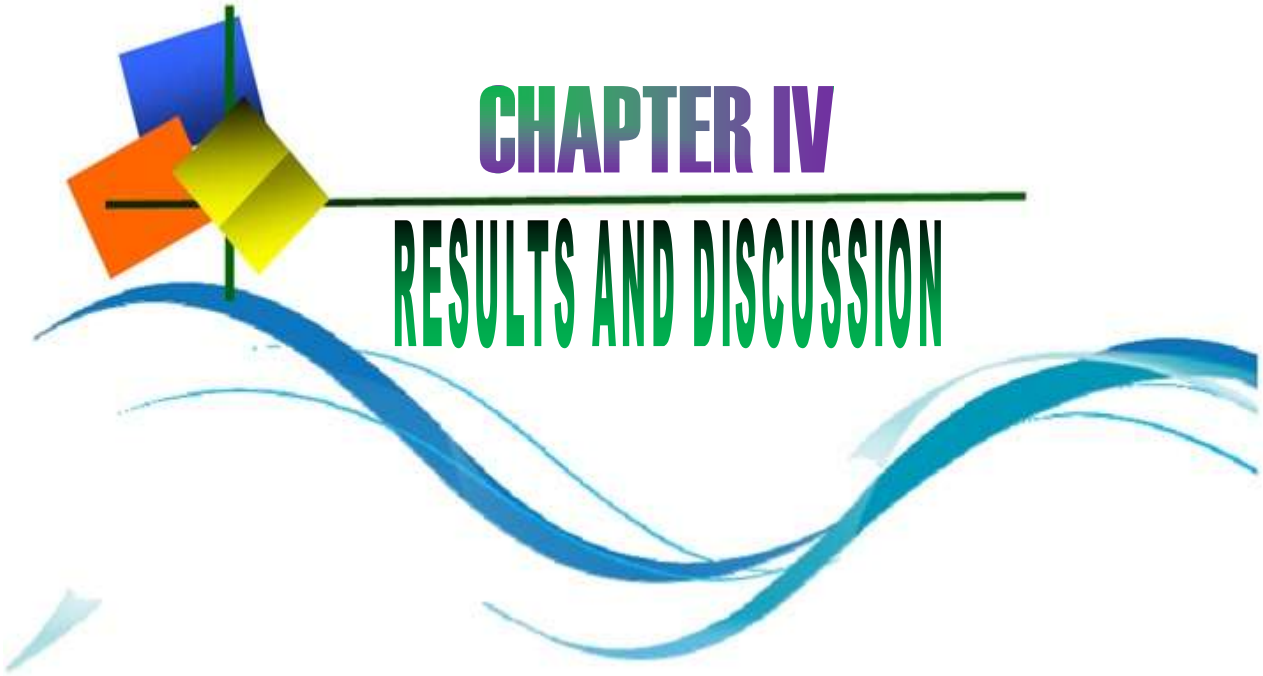
were then categorized according to different class using the following range. Percent repellency > 0.01 to < 0.1 = class 0; 0.1to 20 = class I; 20.1to 40 = class II; 40.1 to 60 = class III; 60.1to 80=class IV; 80.1to100 = class V (McGovern *et al*., 1977)

3.8 Residual or persistence test

The LC_{50} dose of four different solvent extracts of *A. vera* leaves were applied on bean leaf disc (3 cm diameter) with a hand sprayer. After 1, 24, 48 and 72 h of treatment 20 adult females of *T. urticae* were carefully released on the treated leaf discs. Each treatment was replicated 4 times. Mites were considered as dead when they failed to move even after probed with a brush.

3.9 Statistical analyses

Differences in mortality among treatments were analyzed for one way ANOVA using SPSS program Version 20.0 and means were compared using Tukey's test at $P<0.05$. Probit analysis was used to determine LD_{50} and LD_{90} using Polo Plus software (Robertson *et al*., 2007).



CHAPTER IV

RESULTS AND DISCUSSION

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RESULTS AND DISCUSSION

4.1 Adult mortality of *T. urticae* of four different solvent extracts

Acaricidal activity of four different solvent extracts of *A. vera* leaves were tested against *T. urticae* at four different concentrations (0.25, 0.50, 1.0 and 2.0%). Data presented in Table 1 indicating that, acetone extract of *A. vera* showed strong acaricidal activity against *T. urticae* followed by ethanol, methanol and petroleum ether extracts. At 0.25% concentration acetone and ethanol extracts showed significantly higher acaricidal activity over control but there were no significant differences observed among the extracts. At 0.50% concentration acetone extract exhibit higher mortality of mite which was significantly different from other extracts and control except ethanol extract. At 1.0% concentration mortalities in the four solvent extract treatments increased considerably compared to those at 0.25 and 0.5% concentration and acetone solvent extract was showed highest mortality. At 2.0% concentration acetone extract achieved 94.81% mortality, followed by ethanol (81.82%), methanol (71.43%) and petroleum ether (61.04%) (Table 1). The acetone solvent extract of *A. vera* was found to have good contact acaricidal activity against the *T. urticae* of female adult mites. Wei *et al*. (2011) stated that 24 h after treatment, the acetone extract of *A. vera* leaves showed the strongest acaricidal activity against *T. cinnabarinus* female adults. This findings also supported by Zhang *et al*. (2013), they reported that the acetone extract of *A. vera* has a strong acaricidal activity against *T. cinnabarinus* female. Zhang *et al*. (2017) opined that acetone extract of *A. vera* leaves has acaricidal activity against *T. cinnabarinus* and *P. citri*. Amer *et al.* (1989) revealed that ethyl alcohol and acetone extracts were the most potent extracts against adult females of *T. urticae*.

4.2 Ovicidal activity of four different solvent extracts of *A. vera* against *T. urticae*

Ovicidal activities of four different solvent extracts are presented in Table 2. At 0.25% concentration the highest mortality was found in acetone extract which is significantly different from control. All the extracts showed significant mortality of eggs over control except petroleum ether extract at 0.50% concentration. Acetone extract showed higher mortality of eggs which is significantly different from other extracts at 1.0% concentration and control but no significant difference observed between acetone and ethanol aloe extracts. At 2% concentration acetone extract caused 65.0% mortality followed by ethanol (57.0%), methanol (49.0%) and petroleum ether (40.0%) extracts. The ovicidal activities using acetone extract of the *A. vera* leaves have a good effect against *T.urticae.* Zhang *et al*. (2013) stated that acetone extracts of *A. vera* has higher oviposition activity against *T. cinnabarinus*. Kumral *et al.* also (2009) reported that ethanolic extracts of *D. stramonium* leaves and seeds exhibited acaricidal, oviposition deterrent activities against *T. urticae*. The egg mortality of *T. urticae* using methanol extract of river red gum *Eucalyptus camaldulensis* leaves was 63.26% (Yanar *et al*., 2011). Hussen *et al*. (2006) reported that ethanol extract of *Capparis aegyptia* fruit was the least effective extract against *T. urticae* eggs. Derbalah *et al*. (2013) found that *Nigella sativa* Linn. (Ranunculaceae) (seeds) and *Artemisia cina* L. (leaf) (Asteraceae) extracts were toxic to eggs of *T. urticae* with LC_{50} values of 1850.92 and 2740.42 ppm. 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) 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 *A. vera* to *T. urticae* adults by tropical spray

Aloe acetone extracts showed the highest potent acaricidal activities followed by ethanol, methanol and petroleum ether extracts (Table 3). The LC_{50} values after 24 h for adults were 0.45, 0.67, 0.95 and 1.28 respectively, while for eggs 0.95, 1.41, 2.12 and 3.31 were recorded after 7 days. The slope values of the regression line were 2.46, 1.97, 1.68 and 1.46 for adults and 1.51, 1.48, 1.35 and 1.14 for eggs respectively. LC_{90} values were 1.48, 3.00, 5.51 and 9.72 for adults and 6.68, 10.32, 18.75, 43.67 for eggs respectively (Table 3).

Zhang *et al*. (2013) observed the LC_{50} values of aloe acetone extract against female *T. cinnabarinus* were 0.836 and 0.167 mg/ml for 42h and 72h, respectively. Wei *et al.* (2011) also reported the LC_{50} values of aloe acetone extract against *T. cinnabarinus* were 0.614 and 0.099 mg/ml for 42h and 72h, respectively. Wang *et al.* (2007), tested leaf extracts of *Juglans regia* L. with different solvents and reported the LC_{50} values of 730, 1660, 4960, 7450 and 9910 ppm for petroleum ether, chloroform, ethyl acetate, methanol, and distilled water extracts, respectively.

4.4 Repellent effect of four different solvent extracts of *A. vera* against *T. urticae*

The influence of four different extracts of *A. vera* leaves and doses is presented in Table 4. Repellency rate increased with the increase of doses. Among the extracts ethanol showed the highest repellency effect (95%) followed by acetone (87.5%), methanol (77.5%), petroleum ether (70%) at 2% concentration. Number of eggs laid by females showed a significant reduction as compared to control. For egg laying the repellency effects of acetone, methanol and petroleum ether extracts were statistically similar significantly compared to ethanol. Kumral *et al.* (2009) reported that ethanol extracts

both leaf and seed of *D. stramonium* showed repellent and reproduction inhibition activities against *T. urticae*. Kundu *et al.* (2007) investigated the repellent effect of Biskatali plant extract in two solvents namely ethyl alcohol and chloroform against red flour beetle. They observed that ethyl alcohol and chloroform have repellent effect against *Tribolium castaneum*. Antonious *et al.* (2006) found that ethanol extracts of wild tomato leaf showed strong repellency effect on *T. urticae*. Hussein *et al.* (2006) observed that a higher percentage of repellency was recorded in ethanol extract from *Capparis aegyptia* fruits and leaves (96.42 and 86.67 %) respectively. 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 ethanolic leaf extract was more effective as repellent effect against adult females and immature stage of *T. urticae* followed by acetone, petroleum ether and aqueous extraction. Teklay *et al.* (2012) studied the insecticidal and repellent properties of the extracts of garlic, tobacco, neem and *Aloe vera*. All the plant extracts repelled the insects completely. Mostafa *et al.* (2012) reported that the acetone, methanol and water extracts of mahagoni (*Swietenia mahagoni)* leaf showed the larvicidal, anti-feedant and insect repellent activity against the red flour beetle, *Tribolium castaneum.* Dwivedi and Kumari (2000) who also reported that acetone extract of *T. procumbens, R. communis, A. nilotica, L. inermis* and *D. sissoo* were less potent than petroleum ether extracts whereas *P. hysterophorus* extracts in both (acetone or petroleum ether) solvents exhibited the highest repellent action against the storage insect (*C. chinensis*).

4.5 Residual effects of four different solvent extracts of *A. vera* against *T. urticae*

Residual effects of acetone**,** ethanol, methanol and petroleum ether extracts were presented in Figure 1. In residual test, mortality of *T. urticae* varied appreciable. One hour after treatment acetone extracts show higher mortality (48.75%) followed by ethanol (42.50%), methanol (28.75%) and petroleum ether extract (25.00%). Twenty four hours after treatment, all extracts showed significantly different mortality rates than the untreated control. Acetone *s*howed the higher mortality rate than all other treatments. Although, no significant difference was observed among acetone and ethanol, methanol and petroleum ether extract. The mortality percentage decreased after 48 and 72 hours of treatments.

4.6 Probit regression line

The probit regression line of the four different extracts of *A. vera* leaves against *T. urticae* at 24 h are presented in Figure 2. The insect mortality rates showed positive correlation with the doses at all on treatments. The probit regression line of the *A. vera* solvent extracts showed a clear linear relationship between probit mortality and their log doses and the regression lines become sleeper as doses were increased, as the adults were treated with more toxins for the same period at higher doses. The calculated probit regression equation lines of different extracts against *T. urticae* at 24 h were Y= 1.7286x + 3.2499 for methanol, Y= 2.0771x + 3.2265 for ethanol, Y= 2.5715x + 3.2843 for acetone and Y= 1.5562x + 3.2263 for petroleum ether, respectively. The acetone extracts showed the highest mortality followed by ethanol, methanol and petroleum ether (Figure 2).

Table 1. Adult mortality of *A. vera* leaf extracts at different concentrations recorded 24 h after exposure (Mean ± SE) (%)

Extracts	% Concentrations			
	0.25	0.50	1.0	2.0
Acetone	30.00 ± 4.56a (27.27)	48.75 ± 5.54a (46.75)	82.50 ± 3.22a (81.82)	95.00 ± 3.53a (94.81)
Ethanol	22.50 ± 3.22a (19.48)	35.00 ± 4.56ab (32.47)	66.25 ± 4.26b (64.94)	82.50 ± .22ab (81.82)
Methanol	18.75 ± .26ab (15.58)	28.75 ± 4.26b (25.97)	50.00 ± 4.56c (48.05)	72.50 ± 3.22bc (71.43)
Petroleum ether	17.50 ± 3.22ab (14.29)	23.75 ± 4.26b (20.78)	43.75 ± 2.39c (41.56)	62.50 ± 5.20c (61.04)
Control	3.75 ± 2.39b	3.75 ± 2.39c	3.75 ± 2.39d	3.75 ± 2.39d

Values in the parenthesis are corrected mortality (Abbott's 1925).

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

Table 2. Ovicidal activity of *A. vera* leaf extracts at different concentrations recorded 7 days after exposure (Mean ± SE) (%)

Extracts	% Concentration			
	0.25	0.50	1.0	2.0
Acetone	18.00±4.16a	32.00±1.63a	58.00±5.29a	65.00±4.12a
Ethanol	14.00±4.16ab	22.00±5.29a	46.00±3.46ab	57.00±5.00ab
Methanol	11.00±4.43ab	19.00±6.19a	39.00±3.41bc	49.00±2.51bc
Petroleum ether	11.00±3.41ab	15.00±3.41ab	29.00±3.41c	40.00±3.65c
Control	0.00±0.00b	0.00±0.00b	0.00±0.00d	0.00±0.00d

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

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Table 3. Statistical comparison of LC_{50} values of four different *A. vera* extracts against *T. urticae* adults and eggs

	Acetone		Ethanol		Methanol		Petroleum Ether	
	Adult	Egg	Adult	Egg	Adult	Egg	Adult	Egg
LC_{50} (%)	0.446	0.950	0.667	1.406	0.953	2.115	1.279	3.312
Lower limit	0.375	0.558	0.556	1.122	0.780	1.560	1.002	2.118
Upper limit	0.518	2.328	0.796	1.936	1.209	3.505	1.815	8.100
Slope ± SE	2.459 ± 0.27	1.514 ± 0.20	1.966 ± 0.24	1.480 ± 0.21	1.681 ± 0.23	1.352 ± 0.22	1.455 ± 0.23	1.144 ± 0.22
χ^2 (df)	1.838(2)	2.623(2)	1.470(2)	1.676(2)	0.881(2)	0.077(2)	1.025(2)	0.614 (2)
LC_{90} (%)	1.481	6.676	2.994	10.324	5.518	18.746	9.722	43.667

- The original insect mortality data were corrected by Abbott's (1925) formula before analysis
- χ^2 = Goodness of fit
- df= Degrees of freedom
- The tabulated value of χ^2 is 5.99 (df = 2)

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Table 4. Repellent effects of *A. vera* leaf extracts against *T. urticae* after 24 h of exposure

Solvent	Concentrations (%)	Repellency (%)	Repellency Class	No. of eggs/ female after 24 h	
				Treated	Control
Acetone	0.25	65	IV	2.75bc	43.13
	0.50	75	IV		
	1.0	85	V		
	2.0	87.5	V		
Ethanol	0.25	77.5	IV	1.87c	45.12
	0.50	80	IV		
	1.0	82.5	V		
	2.0	95	V		
Methanol	0.25	45	III	5.18bc	34.43
	0.50	55	III		
	1.0	70	IV		
	2.0	77.5	IV		
Petroleum ether	0.25	45	III	7.06b	41.18
	0.50	52.5	III		
	1.0	62.5	IV		
	2.0	70	IV		
Water		35	II	36.25a	51.00

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

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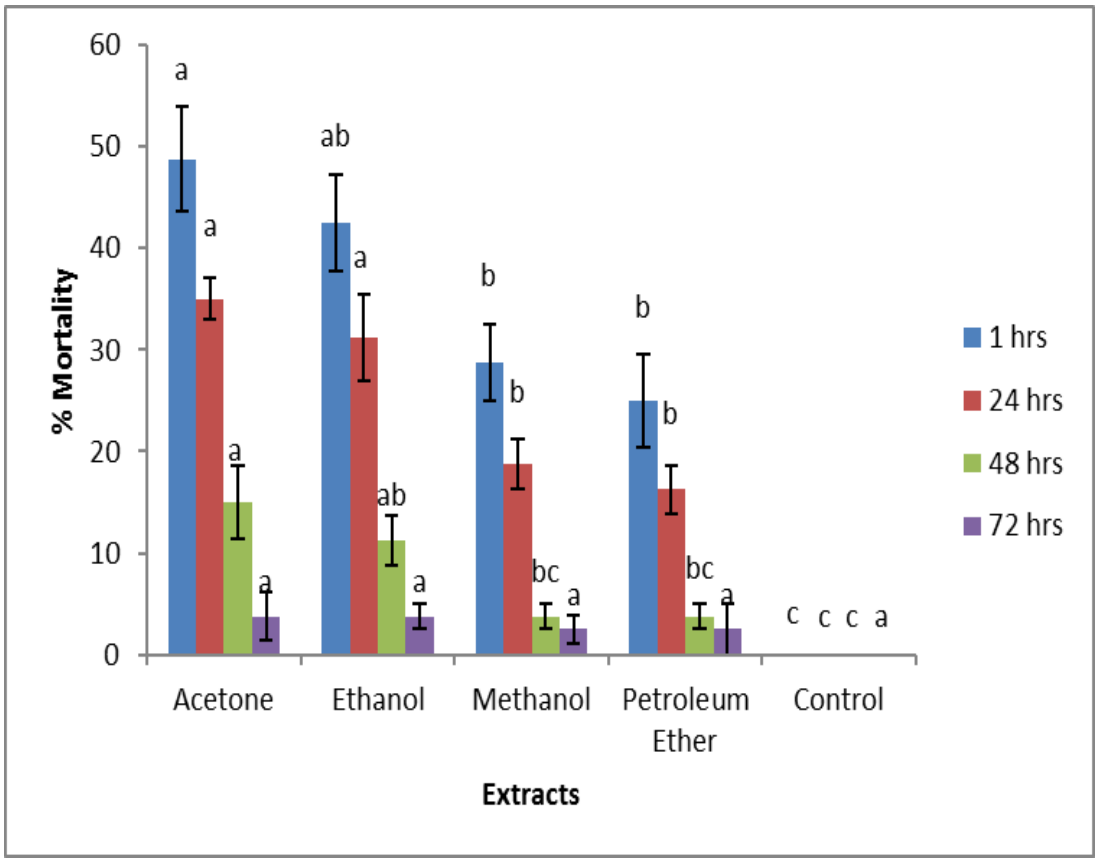

Figure 1. Persistence of *A. vera* leaf extracts against *T. urticae* at 1, 24, 48 and 72 hours old residue of LC_{50} values. [Bars marked with same letter do not differ significantly ($P< 0.05$)]

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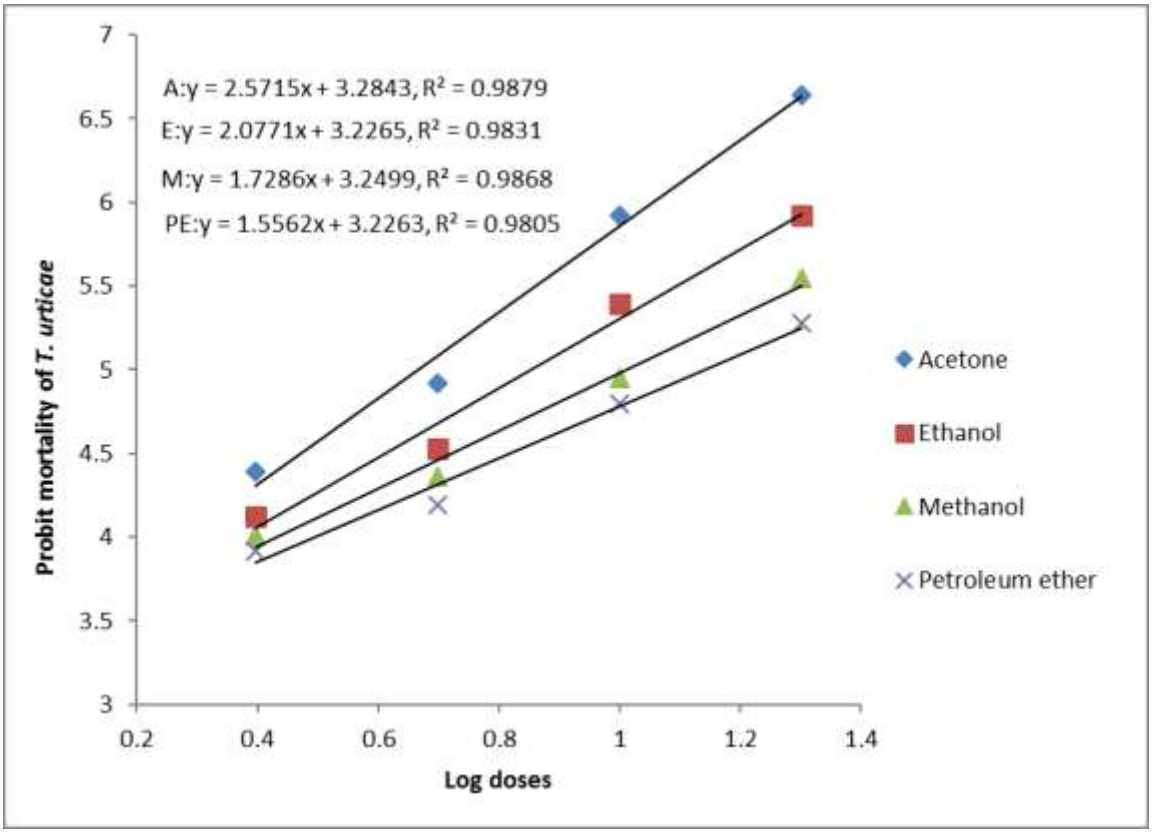
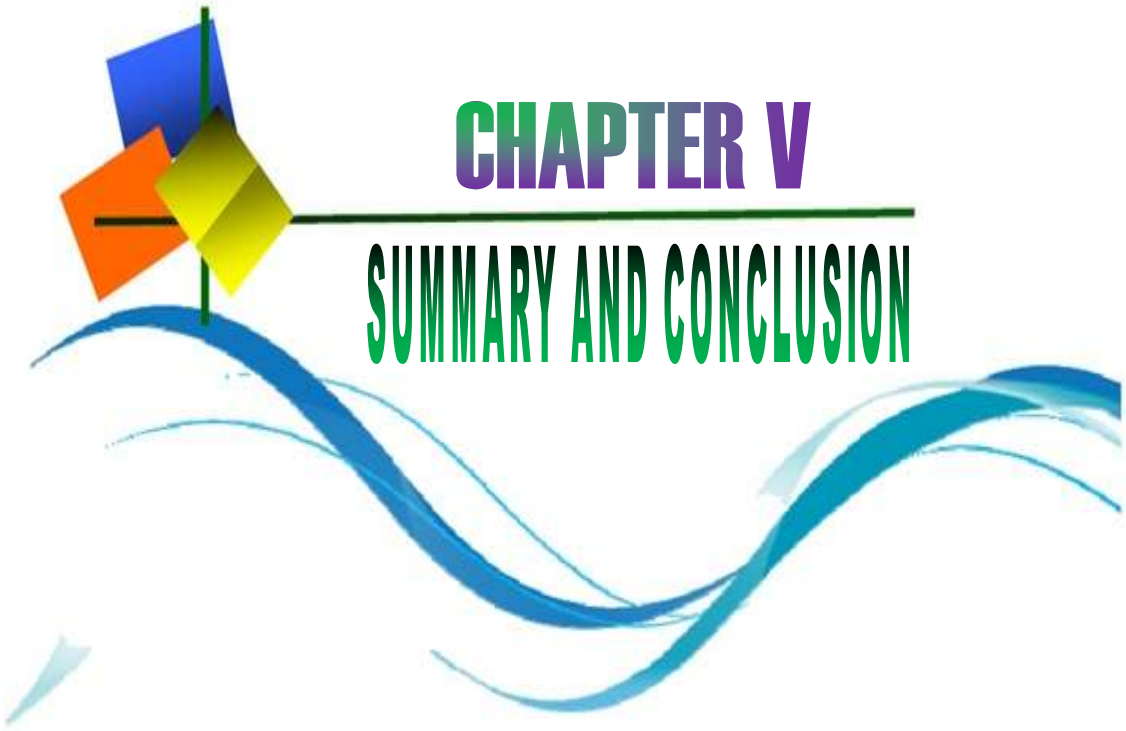


Figure 2. Relationship between probit mortality and log doses of *A. vera* leaf extracts against *T. urticae.*



CHAPTER V

SUMMARY AND CONCLUSION

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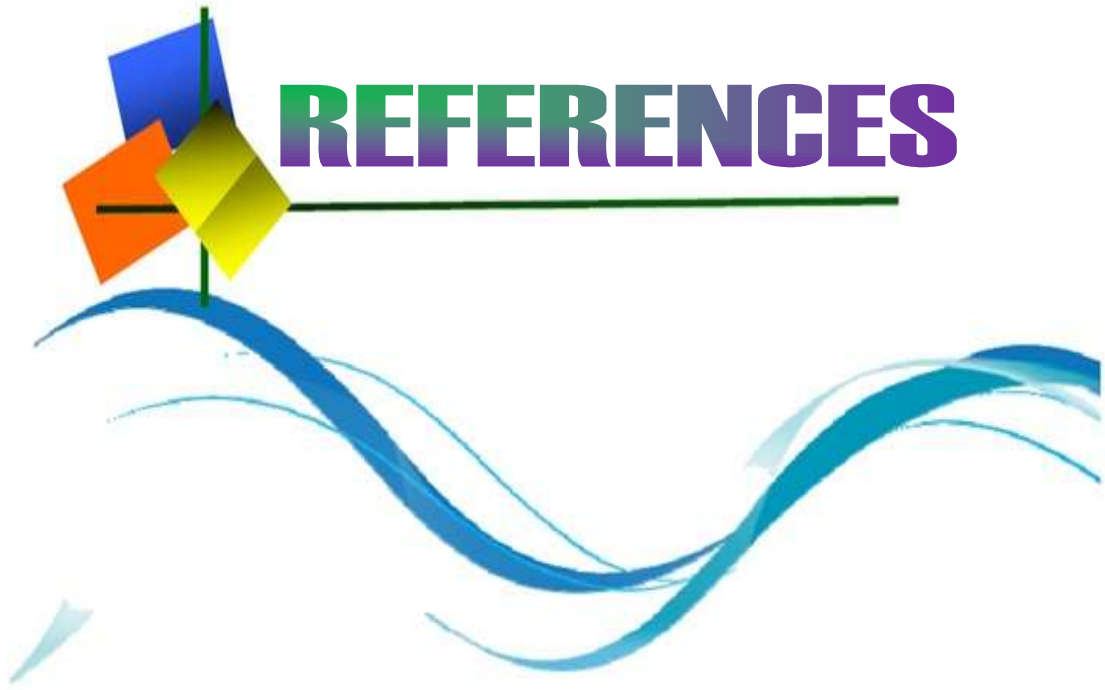
SUMMARY AND CONCLUSION

The present study was undertaken to investigate the effectiveness of *A. vera* L. leaf extract at different concentrations 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 acetone extract possessed the highest mortality (95.00 ± 3.53%) at 2% concentration than other extracts whereas petroleum ether showed the lowest mortality (17.50 ± 3.22%) at 0.5% concentration. Mortality percentage of *T. urticae* egg indicated that acetone extracts possessed the highest mortality (65.00 ± 4.12%) at 2% concentration among all the plant extracts whereas petroleum ether extracts showed the lowest mortality (11.00 ± 3.41%) at 0.5% concentration.

In probit analysis, the lowest LC_{50} value of acetone extract (0.446) indicated highest toxic effects and the highest LC_{50} values of petroleum ether extract (1.279) indicated lowest toxic effects against adult females. In case of egg, the lowest LC_{50} value of acetone extract (0.950) indicated highest toxic effects and the highest LC_{50} values of petroleum ether extract (3.312) indicated lowest toxic effects. The repellency class of different extracts at different concentration level varied between III to V. Water exhibited 35% repellency including repellency class II. Ethanol extract was found more effective as repellent effects against *T. urticae* followed by acetone, methanol and petroleum ether causing reduction in egg production per female by 96, 94, 85 and 83% respectively. The extract also possessed residual effects on this pest. From the result found in residual test, it is found that acetone extract showed highest mortality (49%) at fresh (0 hour) treatment and petroleum ether extract showed lowest mortality (3%) at 72 hour after treatment against *T. urticae*. Therefore acetone extract was found to be most effective to control the *T. urticae* in laboratory condition.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