# COMPARATIVE EFFICACY OF NEEM (*AZADIRACHTA INDICA*) AND AMITRAZ (MITAC) AGAINST THE TWO SPOTTED SPIDER MITES (*TETRANYCHUS URTICAE*), ON TOMATOES (*LYCOPERSICUM ESCULENTUM*).



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## ABSTRACT

Various neem (*Azadirachta indica*. A. Juss) formulations were evaluated under laboratory and greenhouse conditions for their effects on two-spotted spider mites, *Tetranychus urticae* Koch (Prostigmata: Tetranychidae), on tomatoes, *Lycopersicum esculentum*. Four neem formulations (Neemros: neem seed powder with 0.5% azadirachtin, Neemroc: a water miscible emulsifiable concentrate oil, with 0.03% azadirachtin, Saroneem: an alcohol extract; extracted in isopropyl alcohol, containing 1% azadirachtin and Neemroc combi: an enriched oil extract with 0.5% azadirachtin) were tested against two-spotted spider mites. Mitac, an acaricide, was used as a standard in the tests. The effective lethal concentration for each formulation against spider mites was established and compared to Mitac as well as between the neem formulations.

Mitac treatments were more effective than the neem formulations in protecting tomatoes against *T. urticae*. However, Mitac treatments had higher toxicity to predatory mites *P. persimilis* than neem treatments. Among neem formulation, Neemroc EC showed good protection comparable to that of Mitac against *T. urticae*. This treatmemt had a strong feeding inhibition, ovipositional repellence and mortality effect on mites. Saroneem and Neemroc combi gave low protection, although repellence and feeding inhibitions were stronger. Neemros WP was not effective against two-spotted spider mites. Plants treated with Neemros WP, Saroneem and Neemroc combi, had short residual effect compared to Neemroc and Mitac treatments on tomatoes.

A significantly higher tomato yield (weight) was generally observed among treatments compared to the control. Untreated control without mites and Neemroc EC treated plants had higher fruits yield by weight followed by Mitac treated plants. Neem treated plants (Neemros, Saroneem and Neemroc combi) had significantly less fruit yield. Untreated control plants with mites had significantly low fruits yield.

The study generally shows that there is potential for using neem formulations to control two-spotted spider mite in tomatoes. The neem oil formulation Neemroc EC provided better protection almost like that of Mitac.

## DECLARATION

I **SOPHIA SHABAN KASHENGE,** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation has not been submitted for a degree award in any other university.

Signed:.....Kashenge.....

Date:.....12.Nov.1999.....

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## LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| % | percentage |
| $^0$ C | Degrees centigrade |
| AZ | Azadirachtin |
| DAE | Days After treatment Exposure |
| g | gram |
| h | hours |
| Ha | Hectare |
| ICIPE | International Center for Insect Physiology and Ecology |
| IPM | Integrated Pest Management |
| l | liter |
| LDI | Leaf Damage Index |
| m | meter |
| mg | milligrams |
| ml | milliliter |
| MLDI | Mean Leaf Damage Index |
| mm | millimeter |
| N | Nitrogen |
| NO | Neem Oil |
| NSKE | Neem Seed Kernel Extract |
| Ops | Organophosphates |
| RH | Relative humidity |

| | |
|---|---|
| SAS | Statistical Analysis System |
| SE | Standard Error |
| UVPP | Urban Vegetable Promotion Project |
| WP | Wettable Powder |

# CHAPTER ONE

## 1.0 INTRODUCTION

Tomato *(Lycopersicum esculentum* Mill), is one of the world's most popular vegetables. It is probably the most widely grown of all vegetables with an annual world production of 54 million metric tons. Out of this, about 10% is produced in Africa (FAO, 1993). The crop is reported to hold a significant place in the ranking of vegetable crops in Eastern and Southern Africa, surpassed only by brassicas in some countries (GTZ IPM Horticulture, 1994).

Tomatoes are eaten raw in salads, but the bulk is used as flavoring in sauce, stew, and soup and contributes to the value of a meal in respect of carotene, thiamin, niacin, and vitamin C. The fruits are part of every day diet for millions of African households. The leaves are used medicinally for ear-ache and the fruits as a remedy for diseases of the urinary tract (FAO, 1993).

Although the exact record of introduction of tomatoes to Tanzania is unavailable, the crop has been grown in the country for a long time. Tomatoes are reported to have been introduced to East Africa at the beginning of the twentieth century (Groenendijk, 1972).

## 1.1 Importance of Tomatoes in Tanzania

Tomatoes are rapidly gaining position as a staple vegetable fruit due to their culinary uses as salad and dishes among the people of Tanzania. In a survey conducted in 1969, tomatoes accounted for nearly 40% of the 350 000 tons of vegetables consumed in the country (Groenendijk, 1972).

The potential of the crop is generally increasing within the country. A survey conducted in Morogoro region in 1994 showed that an average yield of 10 to 20 tons per hectare was realized by some farmers. This quantity was higher compared to yields of less than 10 tons per hectare before 1994 (Kashenge,1994, unpublished).

The importance of this crop at the national level was realized about 25 years ago (Mlambiti, 1975). The crop is grown for home consumption in backyard gardens of almost every homestead across the country. It is nutritionally important and it also contributes much to the household income. In Morogoro region, tomatoes were a priority crop in 80% of visited villages (Mlambiti, 1975).

Tomato, like most vegetables, is a labour intensive crop. In the African cultural context, women mostly grow the crop. Therefore, it provides employment and income and contributes to food security for large numbers of rural populations.

Despite the difficulties associated with growing tomatoes in the tropics, production increased in Tanzania and in a number of other tropical countries (Tindall, 1983).

The increased awareness of the importance of tomatoes and other vegetable crops in East Africa is due to three main factors. Firstly, because of the nutritional value of the crops, they are being encouraged. Secondly, the export potential for horticultural crops is immense. Not only could these crops be exported to neighboring countries, but also to Europe. Thirdly, climatic conditions in East Africa have been reported to favor the production of these crops all the year around (Nganga, 1971). Experience in Tanzania has shown that a wide range of horticultural crops could be grown particularly due to a wide range of suitable agro-climatic zones.

Tindall (1983) reported that, the potential for tomatoes in Tanzania and other tropical countries is great, but research on the crop has been largely neglected (Swai, 1995).

## 1.2 Tomato Production Constraints in Tanzania

Production of tomatoes per hectare has remained far below its potential. Yields as low as 3 tons per hectare have been reported in Morogoro region

(Kashenge, 1994, unpublished). Average yields of 7 tons/ha in Tanzania (Swai, 1995) and 10 tons/ha in Uganda (Mwaule, 1995) have been realised, although the potential yields of 20 to 30 tons per hectare have been reported else where in East Africa (Nganga, 1971). Varela (1995) reported yields as high as 100 tons/ha in commercial farms in Zimbabwe. Generally, factors that contribute to yield losses include (a) disease and pest outbreaks, (b) seasonal climatic changes, (c) lack of proper management practices, (d) unavailability and/or high prices of inputs, (e) inadequate production techniques and (f) poor seeds (William *et al*, 1991). A survey carried out in rural areas in Morogoro region – Tanzania showed that pests and diseases were the second main constraints in tomato production (Kashenge, 1994, unpublished).

### 1.2.1 Arthropod Pests of Tomatoes in Tanzania

According to Bohlen (1978), the most prevalent and damaging pests of tomatoes in Tanzania are: two spotted spider mites (*T. urticae),* American boll worm *(Helicoverpa amigera* Hübner*),* green peach aphids (*Myzus persicae* Sulzer), tomato erinose mite (*Aculops lycopersici* Messee), white fly (*Trialeurades vaporariorum* Westhood), cutworm (*Agrotis segetum* Denis and Schiffermuller) and nematodes (*Meloidegyne javanica* Treub and *M. incognita* Kofoid and White).

Spider mites (Family: Acarina) are the most difficult pest to control, as they quickly become resistant to synthetic acaricides (David and Brown, 1977). This pest has been reported as a major problem in other countries of Eastern and Southern Africa (Malawi, Zambia, Zimbabwe, Botswana, Mozambique) and some parts of South Africa (GTZ IPM Horticulture, 1994). In Tanzania, the importance of red spider mites is probably vastly underestimated because there have been no studies on the effect of this pest on tomato production.

#### 1.2.1.1 Economic Importance of Spider Mites in Horticulture

Spider mites (Acari: Tetranychidae) are a serious problem in agriculture. The common red spider mites (*Tetranychus cannabarius*), and the two spotted spider mites (*T. urticae*), are major pests of fruit trees as well as greenhouse and field crops (Mansour and Ascher, 1984). Their economic importance is constantly increasing due to their high ability to develop resistance to acaricides and their resurgence after the application of non-selective synthetic pesticides. The latter are harmful to potential natural enemies (Mansour and Ascher, 1984). The application of synthetic pyrethroids against other pests has served to exacerbate the frequency of mites outbreaks (Van de Vrie, 1985; Löhr and Michalik, 1995).

## 1.3 Use of Botanical Extracts

Continuous use of synthetic pesticides has created serious problems due to contamination of the biosphere and poisoning of human beings and arthropod natural enemies (Khorkhordin and Mironover, 1994). As a result, for a long time, efforts have been made to develop alternative pest control strategies to replace synthetic pesticides. Among the methods/techniques which have the potential to replace synthetic pesticides is the use of botanical materials with pesticidal effects (Stoll, 1995). Botanical extracts with bioactivity against anthropoids have several advantages:

- they have, in general, a higher degree of safety to human beings and domestic animals.
- they break down without leaving toxic residues.
- they are generally more environmentally friendly and
- can be less costly.

The use of botanical pesticides that are relatively harmless to natural enemies, could increase the effectiveness of natural predation, thereby reducing the population of pests like spider mites. This may in turn lead to fewer pesticides application, lower production costs and reduced environmental pollution (Mansour and Ascher, 1984).

### 1.3.1 Neem Extracts

Intensive studies on products of the neem tree, *Azadirachta indica* A. Juss, *(syn Anthelaea azadirachta, Melia azadirachta)*, have indicated that many parts of the tree can produce materials for pest control (Jacobson *et al.*, 1984; Schmuterrer, 1990). Although there have been several studies on the efficacy of neem extracts on insects (Schmutterer, 1995), few studies on the effects on two spotted spider mites are documented.

## 1.4. Objectives

### 1.4.1 Broad objective

This study was undertaken to compare the efficacy of commercial neem products with that of a synthetic acaricide (mitac) against the two sopotted spider mites, *T. urticae* on tomatoes.

### 1.4.2 Spercific objectives

1.4.2.1 To detemine the effective concentration of neem formulations against *T. urticae.*

1.4.2.2 To compare the efficacy neem formulations with that of Mitac.

1.4.2.3 To compare yield of tomato plants treated with neem formulations and plants treated with mitac.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Two Spotted Spider Mite, *Tetranychus urticae* Koch: (Prostigmata: Tetranychidae)

Tetranychid mites are found throughout the world on every major food crop and most ornamental plants. The subfamily Tetranychidae includes a number of economically significant species, of which *T. urticae* is the most important in vegetables like tomatoes, beans and cucurbits. These mites occur in several colour forms, including green and carmine form, which were formally known under the names *T. urticae* (two spotted spider mites) and *T. cinnabarinus* (common red spider mite), respectively. These names and colour forms have been the subject of considerable speculation and confusion over the last 40 years (Meyer, 1996).

### 2.2 Pest Status

Two spotted spider mites rank first among the pest problems of tomato almost throughout Southern Africa (GTZ IPM Horticulture, 1994). However, there is very little published information about actual yield losses caused by this pest. Yield increase of between 30 and 80% were reported in plots with weekly acaricide applications as compared to no mites control in Zambia

(GTZ IPM Horticulture, 1994).

## 2.3 Host Range

Two spotted spider mites are known to be the most polyphagous species of the tetranychids. They are major pests of vegetables, ornamentals in greenhouses, in field crops and are also found in forest nurseries (Pritchard and Baker, 1955; Hussey *et al.*, 1969; Jeppson *et al.*, 1975; Plaut and Monsour, 1980; Van der Vrie *et al.*, 1985; Meyer, 1996). These mites have been recorded on more than 200 plants in southern Africa alone (Meyer, 1996).

Spider mites feed primarily on mature leaves by feeding beneath the epidermal layer of cells. They are capable of removing cellular contents, causing cell destruction and reducing photosynthesis. Complete mesophyll collapse and consequently leaf drop can result when plants are stressed by high spider mites infestations. The first symptoms of injury are chlorotic stipples on the leaves; larger areas subsequently turn yellow and leaves become convex. Fine webbing is clearly visible when infestation is severe (Meyer, 1996; Charnie *et al.*, 1998).

## 2.4 Bio-ecology of *T. urticae*

Optimum temperature for the development of two spotted spider mites is between 26°C and 30°C. Mites flourish at low relative humidities. Under

optimum conditions, the eggs hatch after 3 to 4 days. Initially, the larva is orange, but turns green after feeding. When 3 or 4 days old, the larvae undergo a short quiescence to pronymphs, which molt to deutonymphs. After 2 days the deutonymphs undergo a period of quiescence before the adults emerge (Gerson, 1992).

The life cycle from egg to adult takes 10 to 14 days under favourable conditions (Charnie *et al.*, 1998). Extremely high humidities and low temperatures can induce diapause (Sebels, 1981). Under relatively warm conditions the mites reproduce throughout winter and up to 20 generations may occur in the field in South Africa (Charnie *et al.*, 1998). Twenty-four hours after emergence, the female begins to lay eggs and can lay 100-150 eggs in 20-30 days (Wrensch, 1985).

Two spotted spider mites feed and breed throughout the year, except in extremely cold weather where they remain quiescent in winter and hibernate on the ground, under leaves, in cracks, crevices and other sheltered places. Mites are most numerous in hot, dry weather, with populations often declining after rains (Matthew, 1985).

## 2.5 Dispersal Mechanism

Spider mites have well-developed dispersal mechanisms, enabling them to spread over large areas and to colonize widely separated host plants. *T. urticae* is able to crawl over the soil surface to infest neighboring plants. Spider mites are also carried by the wind after first assuming a dispersal posture which involves raising the forelegs upright. All active stages except adult males display dispersal posturing (Kennedy and Smitley, 1985). Under high-density conditions, individuals undergo a change in behavior pattern, which leads to dispersal from the plant. Hussey *et al.* (1969) showed that *T. urticae* has three different methods of spreading. These are: migration of the female to reproduction sites, migration from heavily infested crop by dropping off and migration in accordance with the plane of polarized light.

The dispersal phase of *T. urticae* manifests positive phototactic response. The initiation of the dispersal phase appears to be a response to food shortage and desiccation. The response is intensified under conditions of low relative humidity in the plant micro - climate, as a result of extensive mite feeding on the foliage (Suski and Naegele, 1966). This phototactic response results in dispersal phase, mites moving up the plant and concentrating around the periphery of their host, where, presumably, they are more exposed to wind which leads to their aerial dispersal (Suski and Naegele, 1966).

Hussey and Pall (1963) reported that mites moved up the plant and began to abandon the host when all the apical foliage became damaged. They also observed masses of dispersing mites forming at the apices of foliage and dropping from the plant on webs. This "roping" or spinning occurs only in still air. Mites leaving the plant in this way presumably crawl in search of another plant once they reach the ground. Charnie *et al.*, (1998) reported that wind plays an important role in the dispersal of red spider mites. As a consequence, other crops, wild plants or weeds can serve as a source of infestation. Dispersal among Tetranychidae is a significant factor in their importance as agricultural pests.

## 2.6 Population Development

Population increase of *T. urticae* is determined by many factors including the rate and duration of egg laying, rate of development, sex ratio, host plant conditions, and abiotic factors such as temperature, light, rain, humidity and wind (Van de Vrie, 1985). The potential of increase in a population can be estimated in terms of the intrinsic rate of natural increase ($r_m$) of which fecundity, hatchability, length of oviposition period, longevity, rate of development, survivorship and sex ratio are major determinants (Wrensch, 1985).

The highest $r_m$ is found in the Tetranychini. Species of the genus *Tetranychus* appear to be the most prolific (Van de Vrie, 1985). From 5 days of adulthood

onwards, the female lays up to 10 eggs a day. An increase in $r_m$ leads to an increase in the number of annual generations (Wrensch, 1985). Saito (1979) reported that the higher $r_m$ of the *Tetranychus* species is due to the ability to produce a large number of offsprings under marginal conditions and successful adaptation to an originally unsuitable habitat.

## 2.7 Economic Importance of *T. urticae*

Spider mites are reported to be umbiquitous pests of crop plants. The attack can result in poor crop performance, poor yield and sometimes total crop loss (Meyer, 1981). Being polyphagous, weeds, garden plants, hedgerow plants and trees serve as a source of infestation of red spider mites to the cultivated crops (Hall and Thacker, 1993). Mites feeding lead to leaf color changes, reduction in growth rate, reduced flower formation and yield. These are external symptoms resulting from mechanical damage and biochemical alteration of the plant. As a consequence of damage to plant tissue and disturbance of plant physiological processes, changes in growth intensity, flowering and yield occur (Van de Vrie *et al.*, 1985).

Van de Vrie *et al.* (1985) reported on the slow rate and the delayed effect in the rate of growth of plant stems. They also observed a decrease in number of infested plant leaves through defoliation, early dropping of immature fruits and later ripening of developed fruits. Reduction in the thickness of damaged leaves

has also being reported, resulting from lower number of cells in the leaf (Mothes and Seitz, 1984).

Sabelis (1985) found that over - wintering and diapause of mites under rough bark scales, crevices, ground litter and rubbish contributes to the difficulty in controlling them. Meyer (1981) remarked that their feeding on the lower side of leaves and intense webbing characteristics of high densities increase the difficulties to achieve adequate coverage and hence control with acaricide, contributing to the development of a resistant population. Direct stimulation of reproduction and reduction in interspecific competition induced by dispersal also increases their economic importance in agriculture (Hall and Thacker, 1993).

## 2.8 Damage of Tomatoes by Spider Mites

Mite-induced physiological effects may reduce tomato plant yields by reduced size and number of fruit, and by sunscalded fruit arising from loss of leaves. Stacey *et al.* (1985) found that when tomato plants at different stages of plant growth were subjected to *T. urticae* damage, of all leaves on the plant, the top twelve leaves contributed mostly to yield.

Damage to leaves near a truss of setting fruit is likely to cause yield loss at a rate approximatelly equal to the proportion of leaf area affected: when 10% of

leaf area was damaged, 9% loss in yield resulted (Stacey *et al.*, 1985). Helle and Sabelis (1985) reported that on a scale of 0-5-leaf damage, an index of 2.0 was equivalent to approximately 30% damage of the photosynthetic area of the leaves.

## 2.9 Management of Two Spotted Spider Mites

### 2.9.1 Cultural Control

Several cultural methods have been tried to control spider mites. They include uprooting and burning of old crops, crop rotation and proper field sanitation. These approaches disrupt the life cycle of tomato pests, and reduce the pest population including mites (Tindall, 1983 and Villareal, 1980). However, the majority of farmers do not practice the recommended cultural methods for quality tomato production due to lack of information and knowledge.

In Finland, Tulisalo (1974) developed a program of water sprays in form of mist to inhibit mites infestations. He reported that, in high relative humidity, the females had a shorter life span and laid eggs at a slower rate. Tulisalo (1974) also found that water caused death by suffocation, but to obtain a maximum effect, surface-active agents must be added to remove the thin layer of air surrounding wet individuals.

Many workers have demonstrated the effect of plant nutrition on spider mites,

and therefore, there have been several attempts to reduce mites build up by manipulating plant nutrition. For example, by varying the fertilizer regimes (Markkula and Tittanen, 1969). Large quantity of nitrogen or deficiencies of potassium increase the amount of nitrogen in the plant. Under these conditions there will be a sharp increase in the rate of *T. urticae* population growth. (Watson, 1964; Markkula and Tittanen, 1969). Suski and Badowska (1975) studied mites reproduction on *Phaseolus vulgaris* plants treated with varying rates of nitrogen and found that high doses caused the highest innate capacity for increase.

### 2.9.2 Biological Control

Both indigenous and exotic natural enemies of spider mites can be used as biological control agents. Charnie *et al.* (1998) reported the use of natural biological control and induced or classical biological control where man applies predatory agents. Meyer (1996) found that biological control is poorly applied in tomato production and that fewer natural enemy of red spider mites are found in commercial tomato plantings because of the numerous spray applications for the control of pests and diseases. The following natural enemies species occur in association with *T. urticae*: *Anystis baccarum* (Linn.), *Chaussieria venustissima* (Barelese), *Rubroscirus rarus* (Cunaxidae) and *Eupalopsellus sellnicki* (Eupalopsellidae) (Meyer, 1981).

Hussey and Scope (1985) reported that strains of *Metaseiulus occidentalis* (Nesbitt) are resistant to organophosphorous compounds and carbamate insecticides. Gould *et al.* (1969) reported successful control of red spider mites with *Phytoseilus persimilis* (Athia-Henriot) in greenhouse cucumber. In southern California, weekly releases of *P. persimilis* on infested straw berries over a period of five years at a rate of about five active stages of the mite per plant, effectively controlled *T. urticae*.

On tomatoes, good control has been achieved by introducing ten *P. persimilis* predators to every tenth plant at the first sign of spider mite attacks in the greenhouse (Meyer, 1996).

### 2.9.3 Chemical Control

A wide range of acaricides are registered for use on tomatoes to control spider mites The majority of these can give effective control of all species of spider mites that attack this crop. These include, abamectin, bifenthrin, chinomethionat, cyhexatin, diazinon, dicofol, fenpropathrin, monocrotophos, profenofos and propergite (William *et al.*, 1991, Meyer, 1996). Bohlen (1978) reported the use of azinphos methyl (Gusathion), diazinon, dimethoate and dicofol for the control of mites. Bohlen (1978) also recommended the use of malathion (0.24%), omethoate (0.15%) and parathion (0.2%) for the control of two spotted spider mites.

Kamau (1980) grouped pesticides for controlling mites into three categories depending on effectiveness. These categories were (i) the less effective (malathion, omethoate, dimethoate, and dialifos), (ii) the fairly effective (fenbutatin oxide, micronised sulfur, ethion, azocyclotin and triazine) and (iii) the most effective ( amitraz, endosulfan, binapacryl, dicofol and profenos).

### 2.9.3.1 Resistance of *T. urticae* to acaricides

Resistance of spider mites to acaricides has been reported in various places in the world (Hall and Thacker, 1993; Mansour and Ascher, 1984; Kamau, 1983; Meyer, 1996; Scope *et al.*, 1979; Schulz *et al.*, 1992 and Kleeberg, 1992). The first serious and widespread failure in chemical control was the development of resistance to organophosphates (OPs). It included resistance to parathion and TEPP in 1949-50 only 2-3 years after their introduction in greenhouse crops. However, in fruit orchards, resistance of *T. urticae*, *Panonychus ulmi* and *P. citri* to parathion and subsequently to many other OPs soon became apparent between 1950 and 1960 (Saito *et al.*, 1983).

Busvine (1980) reported resistance of *T. urticae* to funthion, tetradifon, dicofol, binapacryl, carbamates, quinomethionate and cyhexatine. The mite showed strong evidence of multi-resistance to the above chemicals. Hall and Thacker (1993) compared the effect of three permethrin formulations against *T. urticae*. They found that, overall, very few mites died. However, significant treatment

effects were detected in the measurements for repellency, fecundity, and feeding rate.

Charnie *et al* (1998) reported that the major difficulty in controlling an outbreak of spider mites is that they occur on the lower leaf surfaces where they are protected by webbing. Dense foliage and webbing hinders spray penetration and as a result, mites receive insufficient doses required to kill them. Meyer (1981) found that repeated use of an acaricide may kill off the susceptible population leaving eventually only mites that are not killed by the recommended sprays, giving rise to a resistant population.

Resistant populations or strains of mites continue to feed and multiply after application of pesticides that effectively control non-resistant mites. Mites resistant to one acaricide are frequently cross - resistant to other chemically related compounds (Charnie *et al.,* 1998). Resistance of red spider mites develop so quickly that the useful "life" of a new acaricide may be as short as four seasons (Kirby, 1973). It has been reported that resistance problems with phytophagous mites are continuously increasing in the field, greenhouses and orchards (Gunther, 1960; Dancombe, 1973).

### 2.10 Use of Botanical Pesticides

Considerable efforts are being made world wide to find safer, biodegradable

substitutes for the synthetic pesticides (Crombie, 1990). In recent years, studies have focused more towards selective bio-rational pesticides, such as plant - derived compounds because they are generally perceived to be safer than the synthetics (Pimentel *et al.*, 1992). Among these botanicals, azadirachtin (AZ), a mixture of several structurally related tetranotriterpenoids isolated from seeds of neem tree or Indian lilac [*Azadirachta indica* A Juss (Meliaceae)], has attracted the greatest attention in recent years and has been reviewed extensively (Schmutterrer, 1995).

### 2.10.1 The Neem Tree (*Azadirachta indica*)

Neem has been reported to be a fascinating tree (Schmutterer and Ascher, 1984). It seems to be the most promising of all plants as a source of biopesticide and eventually, it may benefit every person on the planet (Schmutterer, 1990). Shultz *et al* (1992) commented that this plant may usher in a new era in pest control, providing millions with inexpensive medicine, cut down the rate of human population growth, and perhaps even reduce erosion, deforestation, and excessive temperature of an overheated globe.

#### 2.10.1.1 Ecology of the Neem Tree

The neem tree is famous for its drought resistance. Normally it thrives in areas with sub-humid conditions, with an annual rainfall between 400 and 1200 mm. It is also grown in regions with an annual rainfall below 400 mm, but in such

areas it depends largely on ground water. Neem can grow in many different types of soils, but it seems to thrive best in well-drained soils. A soil pH value of between 6.2 and 7.0 seems to be best for this tree, but a pH of 5.9 and 10 may also be tolerated under certain circumstances (Schmutterer and Ascher, 1984). Neem is a typical tropical/subtropical plant, which survives in annual mean temperatures between 21 and 32°C. It can tolerate high to very high temperatures. For example, in northeast and central Africa where temperatures can reach 50°C during the summer months. Temperatures below 4°C, and frost, are unfavorable and can result in the shedding of leaves and even death of plants.

The tree is usually found on plain and low-lying hilly land. It thrives at altitudes between 700-800m and occasionally 1000m above sea level. Higher altitude (1000-1500m) are as a rule, much less favorable, with the result that neem trees planted there have a slow growth and low fruit production, owing to cooler temperatures and, often also, high rainfall (Schmutterer, 1995)

### 2.10.2 Pesticidal Effects of Neem Tree Products

#### 2.10.2.1 Antifeedant effect

The neem tree has long been known to be resistant to the attack of many insects. Steets (1976), after reviewing early Indian literature, reported that extracts from neem seeds deter feeding by larvae of sixteen insect species and adults of twelve

insect species in India. It has been documented that neem contains different components with insect antifeeding and growth regulating properties, and yet, it is relatively non - toxic to vertebrates (Butterworth and Morgan, 1971; Steets, 1976; Uebel *et al.*, 1978; Werthen *et al.*, 1978; Jacobson *et al.*, 1978 and Isman *et al.*, 1991). Azadirachtin, salannin and meliantriol have been reported as some of neem components with feeding inhibition properties. Similar observations have been reported for *Panonychus citri* and *T. urticae* (Schmuterrer, 1988; Josh *et al.*, 1984).

### 2.10.2.2 Insecticidal effects

Jaipal *et al* (1984) found that alcohol, ethyl acetate, benzene and petroleum ether extracts of fresh neem leaves gave equivalent toxicity to *Rhyzopertha dominica*. Pure neem oil and its emulsifiable concentrate (EC) formulation have also been found to be insecticidal. The oil had contact toxicity against *Aulacophora foveicollis* (Anon, 1985). The 0.1 and 0.2% emulsions from the EC formulation selectively killed aphids *Rhopalosiphum maidis* (Fitch) and *Melanaphis sacchari* (Zehntner) and spared the larvae and adults of coccinellids and syrphids, the predators of these species (Srivastava and Parmar, 1985).

Schmutterer (1990) studied the efficacy of neem products against *Helicorvepa zea* Boddie, *Spodoptera frugiperda* J. E Smith and *Diatraea saccharalis* in sugarcane. The leaf and seed extracts killed *Heliothis virescens, H. amigera* in

cotton, *Tribolium castaneum* and *Prostephanus truncatus* in maize, *Plutella xylostela*. L, in cabbage, *Empoasca fabae* Harris, *Locusta migratoria* and *M. persicae* (Poswal and Akpa, 1991; Coat, 1994; Yoshida and Tscano, 1994)

Sanguanpong and Schmutterer (1992) confirmed the findings of Mansour and Ascher (1984). They investigated the miticidal potential of a pentane neem seed kernel extracts (NSKEs), and an AZA-enriched NSKE called AZT (9.36%) on *T. urticae*. Female mortality was highest with the pentane extract ($LC_{50}$ = 1.33% in post treatment; 1.82% and 3.25% in pre treatment. The poorest results were obtained with AZT ($LC_{50}$ = 8.86% in post – infestation and 7.17% in pre-infestation treatment).

Neem extracts have also been reported to have acaricidal effects on *T. urticae* Neem extracts repelled approximately 70-90% of female spider mites from treated leaf discs (Schauer and Schmuterrer, 1981; Mansour and Ascher, 1984; Schmutterrer, 1995). Mansour and Ascher (1984) also found that when eggs of *T. urticae,* 24hrs old, were sprayed with methanolic NSKE solution, not only was post-embryonic development retarded considerably, but also mortality set in progressively. However, fewer than 30% of the mites survived and reached adulthood at 20 days after the treatment.

### 2.10.2.3 Oviposition deterrence

The petroleum ether extracts of neem kernel oil were found to be an ovipositional deterrent against *Dacus cucurbitae* Coquillet at 2.5% or higher concentrations and *Dacus dorsalis* Hendel at 20% or higher concentrations (Singh and Srivastava, 1983). Yadava (1985) observed that treating green gram (*Vigna radiata* Wlcz.) seeds with 50mg neem oil / 10g seed prevented oviposition in *Callosobruchus analis* and *Callosobruchus. chinensis*.

A test on the effects of neem extracts on non-target animals, including some arthropods, fish and livestock, indicated excellent selectivity (Schmutterer, 1988). Jacobson (1995) concluded from some of the trials he reviewed that neem products may be toxic to some vertebrates such as birds (chicken), goats, rats and humans. Neem oil, leaf extracts and cake in particular are suspected to contain toxic principals under certain circumstances. Coat (1994) however reported that, residues from neem substances were short lived in the environment.

### 2.10.3 Pesticidal Ingredients of Neem

Neem tree has been reported to protect itself from pests with a multitude of pesticidal ingredients. These compounds belong to a general class of natural products called "triterpenes", and more specifically " limonoids" (Shultz *et al.*, 1992). So far, at least nine limonoids have been reported to have the ability to

block insect growth. They affect a range of species including some of the most serious pests of agriculture and human health. Azadirachtin, salannin, meliantriol and nimbin are the best known limonoids (Shultz *et al.*, 1992).

Azadirachtin is one of the first active ingredients isolated from neem (Shultz *et al.*, 1992). Its major isomer azadirachtin -A [(AZ-A),($C_{35}$ $H_{44}$ $O_{60}$ )], which is present in the extract of neem seeds, highly contributes to the insecticidal activity (Schmutterer, 1990). The Azadirachtin content is between 2 mg/g and 6 mg/g of dried seed kernels (Ermel, 1995). In a survey done over more than 4 years, neem samples from Southeast Asia showed higher content of azadirachtin than neem from Africa. According to Schmutterer (1995) there are differences in the azadirachtin content between trees and locations.

A survey done in Tanzania in 1997 showed that the azadirachtin contents from few plants sampled ranged between 4 mg/g to 7.5 mg/g while the mean value was between 5.2 mg/g and 6.3 mg/g (UVPP, 1997).

## 2.11 Effect of Neem on Predatory Mites *Phytoseiulus persimilis* (Mesostigmata: Phytoseiidae)

Neem products are also claimed to have low toxicity on beneficial insects and insect natural enemies. The use of predatory mites for controlling *T. urticae* has increased considerably since the first trials by Bravenboer and Dosse (1962).

As a bio-control agent, it is used in several countries for the control of spider mites in greenhouses (Vanzon and Wysoki, 1978), and in outdoor crops (Oatman *et al.*, 1976).

The predatory mite, *P. persimilis,* originally from South America, has been consistent and remarkably efficient in the control of spider mites in greenhouses in Great Britain and Europe (Meyer, 1996). However, *P. persimilis* has been reported to be very susceptible to synthetic pesticides (Meyer, 1996), such as organophosphates and synthetic pyrethroid compounds, a situation which complicates its use in Integrated Pest Management (IPM) (Mansour *et al.*, 1986). Although there is plenty of information on the effect of neem products on various crop pests, there is little information documented on the effect of neem on natural enemies, notably predatory mites.

## 2.12 Neem Based IPM Programs

There is overwhelming evidence that pesticides alone do not lead to sustainable pest management in agriculture. The cost of the chemicals and their long term deleterious effects on the environment and health deter their continued use, inspite of earlier spectacular success (Kibata, 1995).

IPM requires the farmer to be knowledgeable about the identity and roles of beneficial insects and other biological control agents, the roles and potential

disadvantages of pesticides use and abuse, and a wide array of cultural and crop sanitation practices that reduce pest incidence (Anon, 1992). Anon (1992) reported on the use of IPM in rice. They compared IPM with synthetic insecticides and IPM with NSKE 5%. The results indicated that use of NSKE 5% spray was as effective as a pesticide application in suppressing insect pests and diseases. In another experiment, Jacob (1989) tested neem oil (NO) and NSKEs either alone or as mixtures, with a synthetic insecticide (monocrophos) for their efficacy in the control of insect pests and diseases of mung bean as part of an IPM programme. NO and NSKE were more effective when used as mixtures with the synthetic pesticides than when applied separately.

IPM has the advantages of reducing the use of synthetic pesticides and the pesticide pressure on beneficial insects and mites. In view of the ever-increasing demands for food, IPM is destined to become a vital part of agricultural strategies in many places.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

The experiments were conducted under laboratory and greenhouse conditions at the International Center of Insect Physiology and Ecology (ICIPE), Kenya. Commercial neem products and a standard acaricide were used. The tomato variety used was MoneyMaker, which is susceptible to two spotted spider mites infestation.

### 3.1 Laboratory Experiments

#### 3.1.1 Neem Formulations

Four commercial neem product formulations were used in the treatments. These were: (i) Neemros (WP), (Neem seed powder with 0.5% azadirachtin), (ii) Neemroc EC oil, a water miscible formulation with 0.03% azadirachtin, (iii) Saroneem (an alcohol extract containing 1% azadirachtin) and (iv) Neemroc combi (an enriched oil extract with 0.5% azadirachtin). Mitac (EC 200g/l amitraz) was used as a standard acaricide.

##### 3.1.1.1 Dilutions

A method developed by Ascher (1981) and Dreyer (1984) was used in the dilutions. Each formulation was diluted to four different concentrations. In each of the tests, there were three replications per treatment, making a total of 15

observations including the control. The control consisted of pure water applied on the leaf discs. The concentrations used for each treatment are shown in Table 1.

Table 1 Neem and mitac dilutions used for determination of effective concentrations against the two spotted spider mites.

| Formulation | Concentration | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Neemros (g/l) | 20 | 25 | 30 | 35 |
| Neemroc (ml/l) | 10 | 15 | 20 | 25 |
| Saroneem (ml/l) | 5.0 | 5.5 | 6.0 | 6.5 |
| NeemrocCombi (ml/l) | 0.1 | 0.5 | 1.0 | 1.5 |
| Mitac (ml/l) | 1.5 | 2.0 | 2.5 | 3.0 |
| Mitac+Neemros (ml+g/l) | 1+20 | 1+25 | 1+30 | 1+35 |

**3.1.1.2 Rearing of Mites**

The strains of spider mites (*T. urticae*) and predaceous mites (*P. persimilis*) were collected from infested leaves of beans, eggplants, and tomatoes and transferred to two different rearing rooms, with controlled temperature (one for two spotted spider mites and another for predaceous mites). Rearing of mites was done on 3-4 weeks old tomato plants in pots (14 ×15 × 8 cm). The stock culture of mites was maintained under controlled conditions in an acclimatized room at 25 - 27°C and 60 ± 5% RH (Mansour and Ascher, 1984).

Mites were transferred from aging plants to the younger ones by placing old leaves infested with mites onto the younger plants. This allowed mites to reproduce continuously for the experiments. Individual mites were collected and transferred for bioassay tests using a fine brush.

### 3.1.1.3 Bioassay Procedure and Determination of $EC_{50}$

The bioassay procedures followed a method described by Ascher (1981).

(i) Tomato leaf discs, 25mm in diameter, were dipped in the test solution of known concentration for 5 seconds and left to air-dry for 1h at 25-27°C. The control discs were dipped in pure water.

(ii) The petri dishes in which the leaf discs were placed were lined with a layer of cotton wool. Tap water was added to wet the cotton up to saturation point. The wet cotton wool was covered with filter paper. One treated leaf disc was placed on the filter paper before introducing fifteen adult female mites. The petri dishes were covered with a lid with perforations for aeration. The experimental conditions were maintained constant at 25 ± 2°C and 60 ± 5% RH.

Number of mites that strayed from the leaf disc (repellence) and number of dead mites (mortality) in each leaf disc were recorded twenty-four hours after treatment. Mites were considered dead when they did not respond to gentle

prodding with a camel hair - brush. The percentage mortality and repellence was calculated and the data were subjected to statistical analysis after being corrected for natural mortality using Abott's formula (Abott, 1925). The effective concentration that killed 50% of mites ($EC_{50}$) was determined for each formulation.

### 3.1.2 Comparison Between Treatments

#### 3.1.2.1 Mortality and repellence of mites treated with fresh residues

Table 2 Dosages of neem formulations and mitac used for treatment of leaf discs.

| Formulation | Dosage |
|---|---|
| Neemros | 30 g/l |
| Neemroc | 20 ml/l |
| Saroneem | 6 ml/l |
| Neemroc Combi | 1 ml/l |
| Mitac | 2 ml/l |
| Mitac | 1ml/l |
| Mitac+Neemros | 1 ml + 30 g/l |
| Control | Untreated |

A leaf disc was dipped in the dilutions (Table 2), for five seconds and left to air dry. For each formulation there were three replicates and a control. Fifteen

female mites of similar age were introduced to each of the treated leaf discs and control. Mortality counts were carried out at 12, 24, 48 and 72 hours post treatment. Repellence was determined by counting the number of mites that strayed off from the disc after the same period of time.

### 3.1.2.2 Antifeedant Effect

Antifeedant effects were investigated using the method described by Busvine (1980). One half of a 25mm-tomato leaf disc was carefully treated and the other half was not. After drying the discs in air, fifteen mites were introduced at the center of each leaf disc. The number of mites feeding on each half of the disc as well as the number of eggs laid after 24 and 72hrs were recorded.

### 3.1.2.3 The Effect of Treatment on Number Pre-imaginal Stages of *T. urticae*

Fifteen female mites were introduced to the treated leaf discs. Counts of eggs and nymphs of mites emerging were recorded after 2, 4 and 6 days.

### 3.1.2.4 The Effect of Treatment on Adult Female Predatory Mites, *P. persimilis*

Five adult female predatory mites *(P. persimilis)* were introduced to treated leaf discs. These were supplied with twenty adult *T. urticae* to feed on. Mortality of the predaceous mites was calculated on the basis of the number of dead females

after 48hrs.

### 3.2 Data analysis

The data were subjected to statistical analysis using the SAS computer program. Comparison between treatments were made and tested for significant difference using Ryan-Einot-Gabriel-Welsch Multiple Range Test (SAS, 1990).

#### 3.2.1 Efficacy of Neem Extract Formulations Against *T. urticae* in the Screen House

Tomato seeds (cultivar: MoneyMaker) were sown in seed trays. About 80% germination had been obtained by the 5$^{th}$ day. One month after germination, 27 seedlings were transplanted in 25 X 32 X 20 cm plastic pots, filled with a mixture of soil, sand and farmyard manure at a ratio of 2:1:1. All plants were staked, trained and pruned to single stem, and watering was done once a day. Calcium nitrate (15.5%) was applied 45 days after transplanting at the rate of 320 kg N/ha. The pots were arranged in randomized manner with three replicates. Each plant was infested with 25 mites at 34 days after transplanting.

The plants were sprayed with the following formulations using a micro–capillary applicator:

- Neemros 30g/l
- Neemroc 20ml/l

- Saroneem 3. 6ml/l
- Neemroc combi 1ml/l
- Mitac 2ml
- Mitac 1ml/l
- 1ml Mitac + 30g/l Neemros
- Control: In one set of control experiments, the plants were infested with mites and sprayed with water. The second set of control experiments was a yield check, in which the plants were neither infested with mites nor treated.

Spraying was carried out at 48, 55, 62 and 68 days after planting. Mites on each plant were counted and a Leaf Damage Index (LDI) was calculated using the method described by Hussey and Scopes (1985). Counting of mites and foliar damage assessment were carried out on the $6^{th}$ day after treatment, and subsequently on intervals of 6 days until crop maturity.

The LDI was established for the ten apical leaves of each plant. Damage was assessed using ratings of 0 to 5, in which:

0 - no damage

1 - the first attack of mites with a few small feeding patches,

2 - large feeding patches <25% leaf area,

3 – feeding patches >25% leaf area,

4 – entire leaf with feeding marks but still green,

5 – necrotic and chlorotic area, the leaf begins to shrivel (Fig 1).

The total fruit yield (weight) and number of fruits per treatment was also determined. For each plant, the Mean Leaf Damage Index (MLDI) was estimated by adding the value assigned to individual leaves, divided by the number of leaves sampled. In addition, all adult mites from 10 apical leaves were counted and recorded. The data were subjected to analysis of variance and means were separated by Ryan-Einat-Gabriel-Welsch multiple range test.

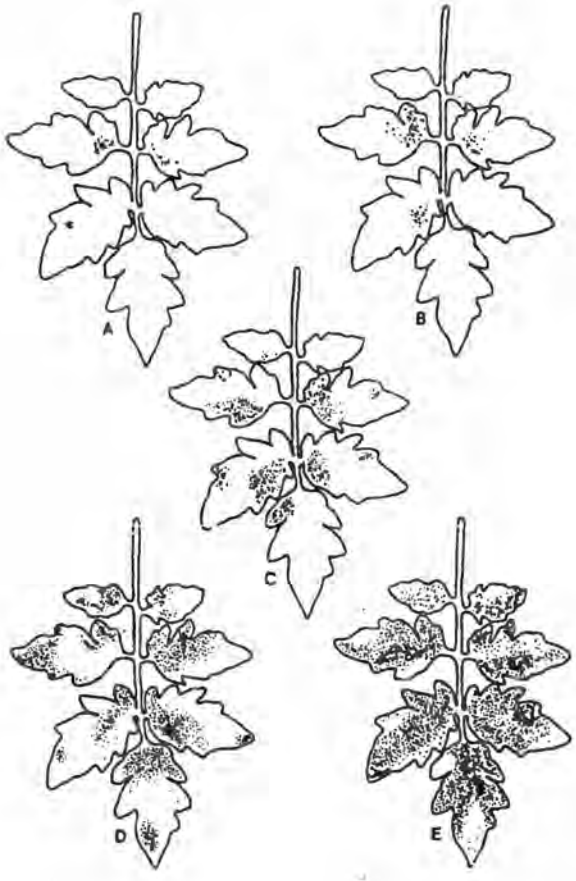


Fig.1. Leaf Damage Index (LDI), as shown by the degree of leaf damage. A = 1; B = 2; C = 3; D = 4; E = 5 (Hussey and Scope, 1985)

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Laboratory Screening of Neem Products and Mitac for Control of *T. Urticae*

#### 4.1.1 Relationship between Mortality/Repellence of Mites and Dosage

The mortality/repellence response of *T. urticae* to different dosages of neem formulations and mitac are presented in Figure 2. The results indicate a strong positive correlation between mortality/ repellence of mites and the applied dosages (Figs. 2a, b, c, d, e and f).

In some of the treatments, there were no statistically significant differences in mortality and repellence between some of the dosages. However, except for the application of Neemros, all dosages differed significantly from the control for both mortality and repellence (Table 3). Neemros WP gave less protection against mites compared to the other treatments. This treatment resulted to lower mortality and repellence at each concentration level compared to Neemroc, Mitac and Mitac+Neemros. Treatment with 35g/l gave significantly higher ($P > 0.05$) mortality, but did not differ significantly from the application of 25 and 30 g/l of Neemros. The repellence caused by treatment with 30g/l was significantly higher ($P > 0.05$) than application of 35g/l , (Table 3). However, the recommended dosage for Neemroc application (30g/l) is lower than the determined $LC_{50}$ (38g/l).

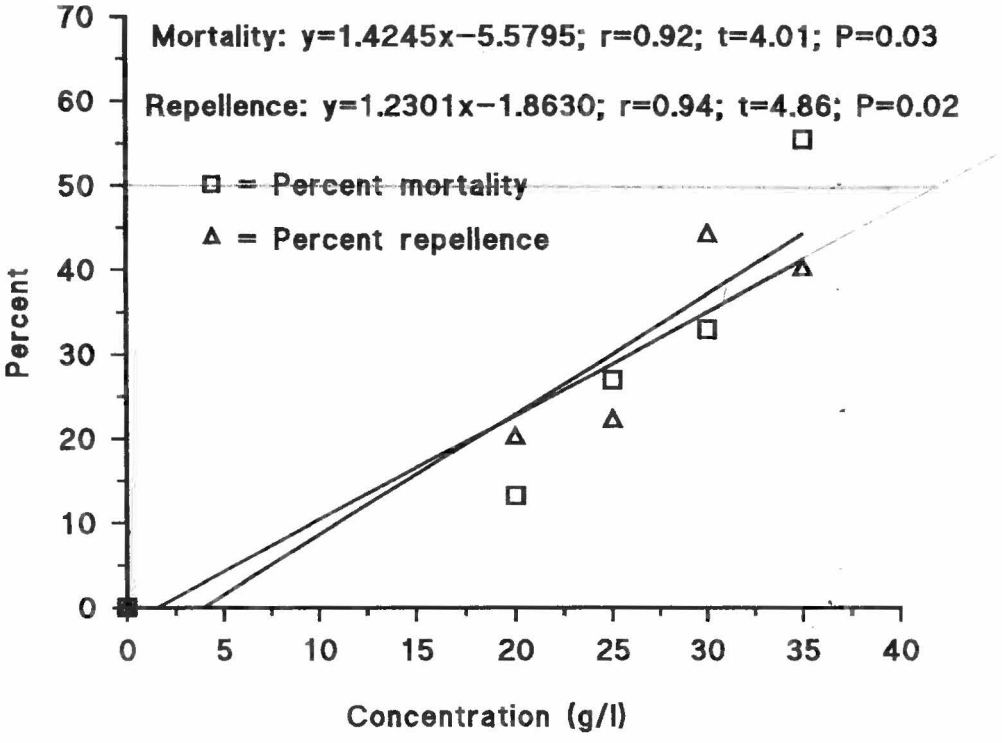


Fig. 2a: Mortality/repellence responce of T. urticae treated with different dosages of Neemros (24h exposure).

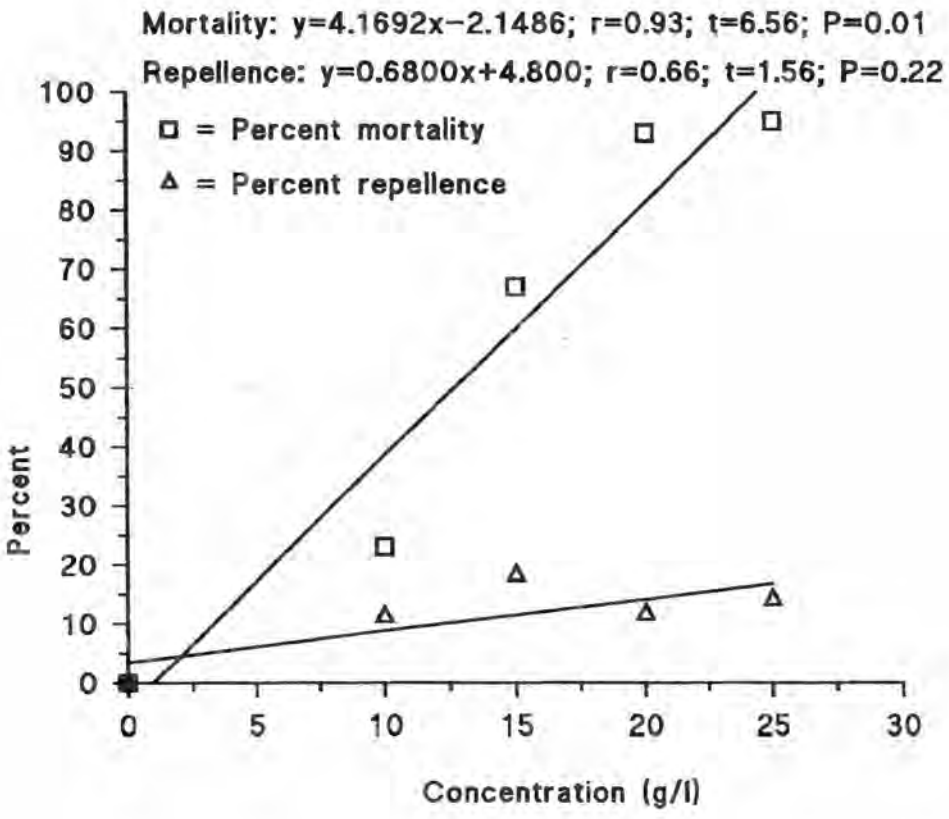


Fig. 2b: Mortality/repellence responce of T. urticae treated with different dosages of Neemroc (24h exposure).

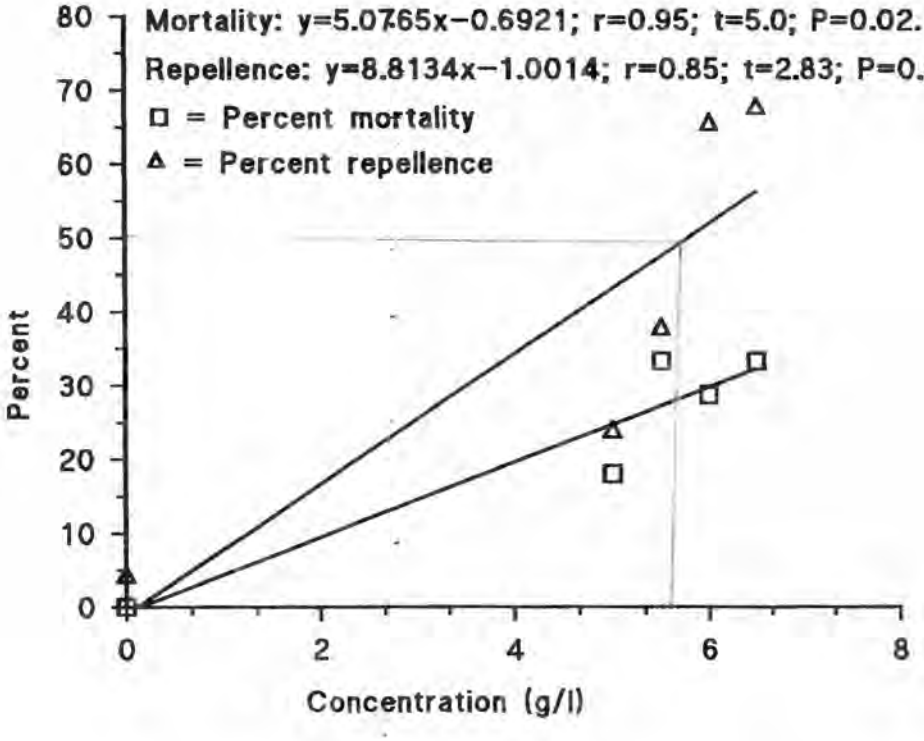


Fig. 2c: Mortality/repellence responce of T. urticae treated with different dosages of Saroneem (24h exposure)

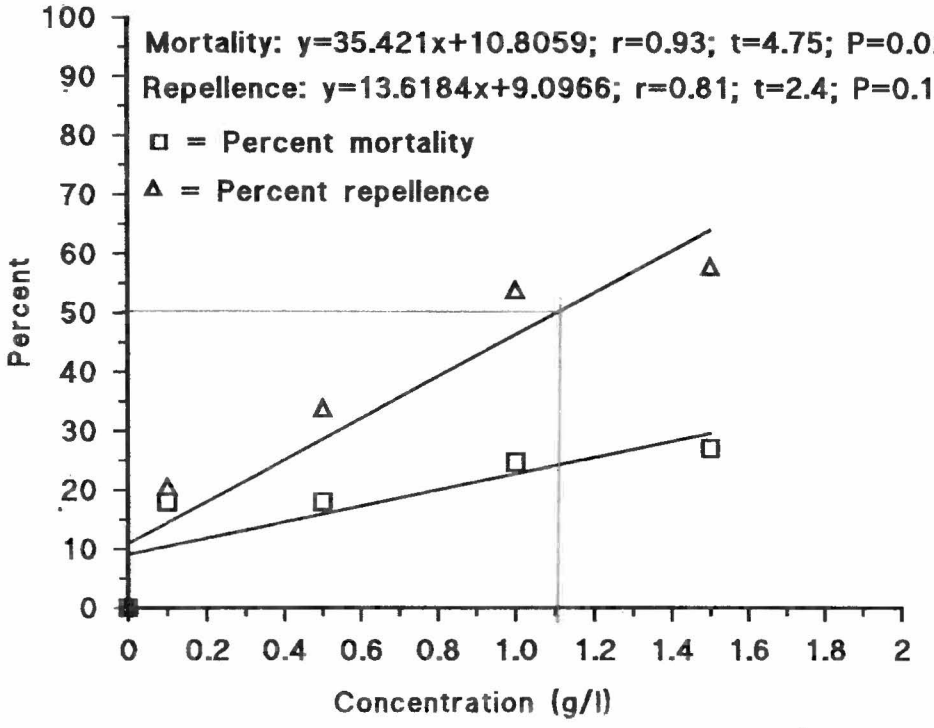


Fig. 2d: Mortality/repellence responce of T. urticae treated with different dosages of Neemroc Combi (24h exposure).

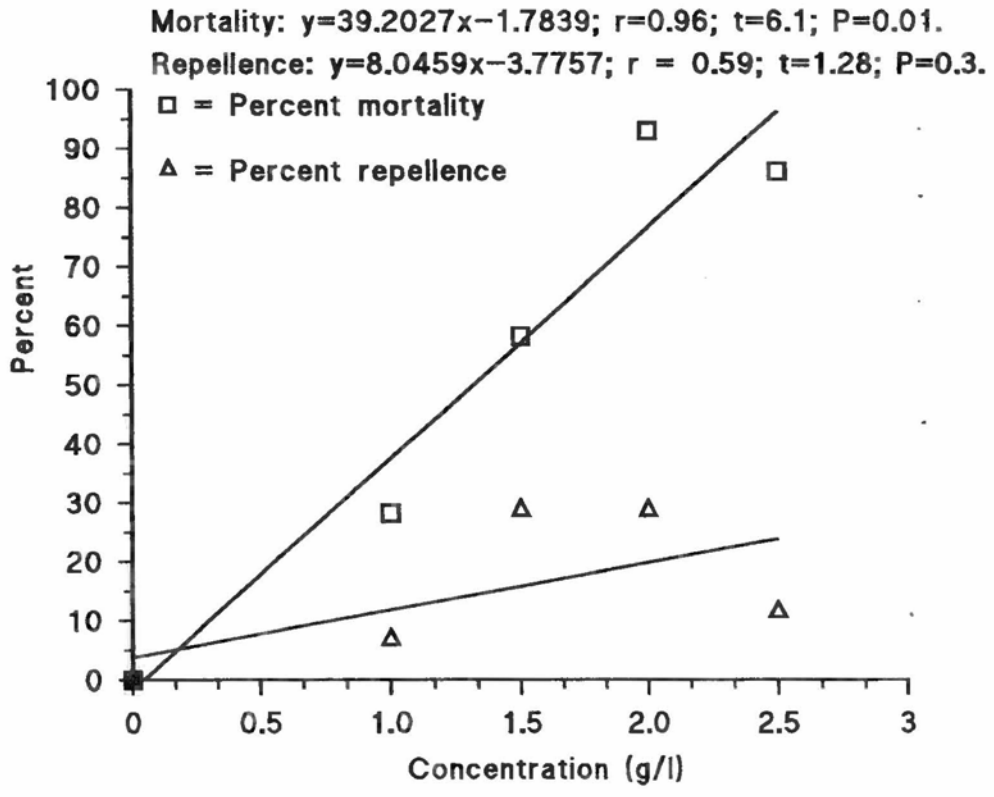


Fig. 2e: Mortality/repellence responce of T. urticae treated with different dosages of Mitac (24h exposure).

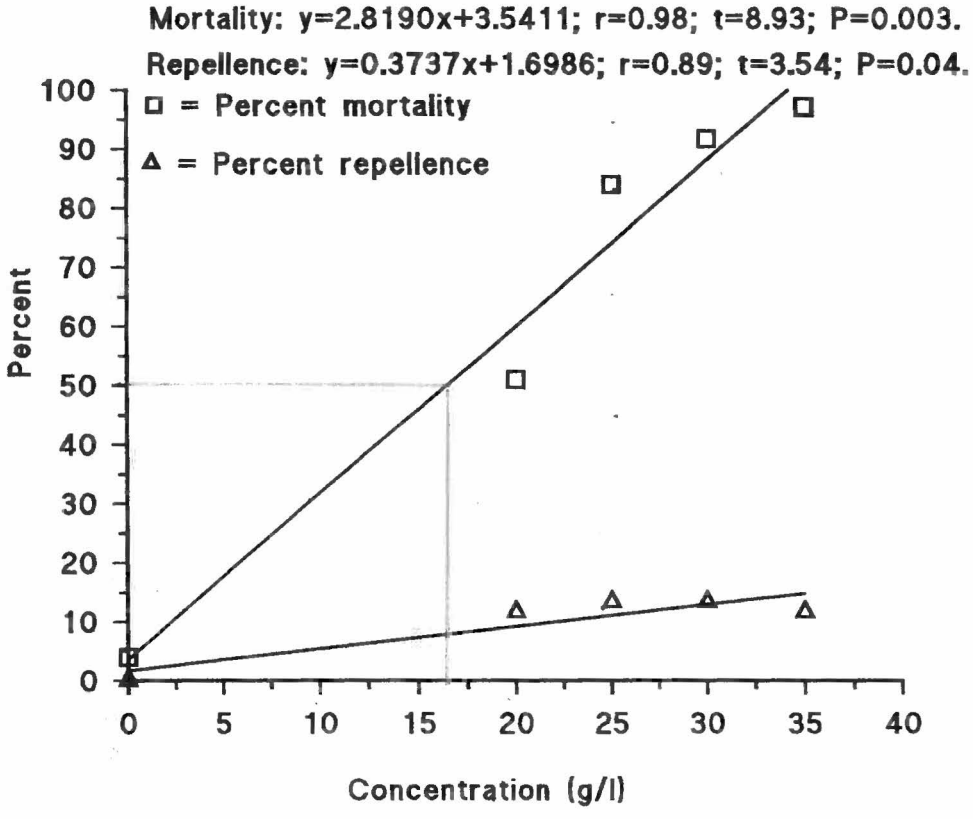


Fig. 2f: Mortality/repellence responce of T. urticae treated with different dosages of Neemros + 1ml of Mitac (24h exposure).

Table 3 Mortality and repellence of two spotted spider mites *(T. urticae)* at different dosages after 24h.

| Treatment | Conc. | %Mortality ([x] mean ± S.E) | %Repellence |
|---|---|---|---|
| Neemros T1(g/l) | 0 | 0c | 0 c |
| | 20 | 13.3 ± 7.7bc | 15.5 ± 5.9bc |
| | 25 | 26.7 ± 7.7abc | 20 ± 3.9bc |
| | 30 | 33.3± 10.2ab | 44.5 ± 9.7a |
| | 35 | 55.6± 8.0a | 28.9 ± 2.2ab |
| Neemroc T2 (ml/l) | 0 | 0b | 0b |
| | 10 | 24.6 ± 11.7b | 13.3 ± 3.8ab |
| | 15 | 65.1 ± 18.4a | 26.7± 10.2a |
| | 20 | 93.2 ± 0.13a | 6.7 ± 0ab |
| | 25 | 95.6 ± 4.4a | 4.4 ± 4.4ab |
| SaroneemT3 (ml/l) | 0 | 0c | 0 b |
| | 5.0 | 23.6 ± 5.3bc | 17.8 ± 5.9ab |
| | 5.5 | 36.6 ± 8.9ab | 33.3 ± 3.8 a |
| | 6.0 | 64.3 ± 9.9a | 28.9 ± 5.9 a |
| | 6.5 | 67.0 ± 6.4a | 33.3 ± 10.2a |
| Neemroc Comb.T4 (ml/l) | 0 | 0b | 0 b |
| | 0.1 | 20.0 ± 7.7ab | 17.7 ± 2.2 a |
| | 0.5 | 33.3 ± 7.7ab | 17.8 ± 5.9a |
| | 1.0 | 53.3 ± 15.4a | 24.4 ± 4.3 a |
| | 1.5 | 57.8 ± 17.4a | 26.7 ± 2.7 a |
| Mitac T5 (ml/l) | 0 | 0d | 0 b |
| | 1 | 25.9± 12.5cd | 28.9± 2.2 a |
| | 1.5 | 56.8 ± 5.5bc | 28.9 ± 2.2a |
| | 2.0 | 92.5 ± 4.4a | 6.6 ± 3.8b |
| | 2.5 | 85.8 ± 10.9ab | 11.1 ± 8.0ab |
| Mitac+NeemrosT6 (ml +g /l) | 0 | 0b | 0 b |
| | 1 + 20 | 63.7 ± 15.6a | 22.2 ± 8.9ab |
| | 1 + 25 | 78.9 ± 4 a | 13.3 ± 3.8a |
| | 1 + 30 | 90.0 ± 4 a | 11.1 ± 5.9a |
| | 1 + 35 | 97.7 ± 2.3a | 6.7 ± 3.8a |

[x] Within columns, means followed by a common letter are not significantly different at P = 0.05 (Ryan-Einot-Gabriel-Welsch Multiple Range Test).

The mortality of mites at a dosage of 10 ml/l of Neemroc EC was low and was not significantly different from the control. The mortality caused by application of dosages of 15, 20 and 25 ml/l did not differ significantly from each other. Treatment with 15ml/l of Neemroc gave highest repellence of mites (Table 3). The lethal concentration ($LC_{50}$) of Neemroc obtained in the current study was 17ml/l, which is lower than the recommended rate of application, (i.e. 20ml/l).

Saroneem at a dosage of 5ml/l gave the lowest mortality, which did not differ significantly from the control. There were no significant differences ($P > 0.05$) in mortality of mites at dosages of 5.5, 6.0 and 6.5ml/l of Saroneem (Table 3). All dosages of Saroneem gave significantly higher repellence levels than the control. The $LC_{50}$ (5.9 ml/l) estimate was similar to the recommended application dose (6.0 ml/l).

Treatments with Neemroc Combi at dosages of 0.1 and 0.5ml/l were not significantly different ($P > 0.05$) from the control. However, dosages of 1.0 and 1.5ml/l gave significantly higher mortality ($P < 0.05$) than the control (Table 3). The recommended concentration for Neemroc Combi (1 ml/l) was the same as the $LC_{50}$ (1 ml/l) estimates obtained in this study. The mortality caused by Mitac at a dosage of 1.0ml/l was not significantly different from the control. At dosages of 2.0 and 2.5m/l, relatively higher mortality was obtained but did not differ significantly from one another. The $LC_{50}$ estimate (1.49ml/l) was lower

than the recommended dosage for mitac application (2ml/l). Mixtures of Mitac and Neemros applied on leaf discs did not produce significant differences in mortality of mites. However, the mortality was significantly different ($P < 0.05$) from the control. Similar observations were made for repellence of mites (Table 3), in which the $LC_{50}$ estimate was 1ml of Mitac when mixed with 25g Neemros/l.

### 4.1.2 Mortality and Repellence of Female *T. urticae*

#### 4.1.2.1 Mortality

The mortality of *T. urticae* increased with time in all treatments (Fig. 3a, b, c and d). At 12h after exposure, all the treatments except Neemros gave higher mortality than the control. Neemroc, Mitac 2m/l, Mitac 1m/l and Mitac+Neemros treatments gave higher mortality of mites than Neemros, Saroneem and Neemroc Combi (Fig. 3a). In general, there were minor variations in mortality of mites after 24h exposure to different formulations of neem (Fig 3b, c, and d).

#### 4.1.2.2 Repellence

Generally, repellence of mites increased with time (Fig 4a, b, c, and d). There were no significant differences of repellence in different treatments after 12h (Fig.4a). After 48h of exposure to treated discs, Neemroc combi and Mitac+Neemros gave significantly higher repellence than the control (Fig. 4c).

At 72h, Saroneem, Neemroc combi, and Mitac treatments gave significantly higher repellence than the control. Saroneem and Neemroc Combi also gave higher repellence than the other formulations.

### 4.1.3 Effect of Neem Formulations and Synthetic Acaricide on Feeding and Oviposition

#### 4.1.3.1 Feeding Deterrence

Most mites moved to the untreated sides of the leaf discs (Table 4). Generally, the number of mites on treated side of the leaf disc was significantly lower than on untreated side. Neem formulations gave higher feeding deterrence than Mitac treatments.

Feeding was almost completely inhibited within 24h when the leaves were treated with Neemroc, Saroneem and Mitac + Neemros. However, at 72h, feeding inhibition by Saroneem and Mitac + Neemros decreased while that of Neemroc remained the same. When mitac was applied together with Neemros, feeding inhibition increased to a level higher than that of Neemros and Mitac applied separately.

#### 4.1.3.2 Oviposition Deterrence

Mites preferred to lay eggs on untreated sides of leaf discs, indicating oviposition deterrence by neem treatments. There were significant differences

between treated sides and untreated sides of leaf discs at both 24 and 72h post treatment. Except for Neemroc combi, oviposition deterrence increased with time in all treatments, (Table 5).

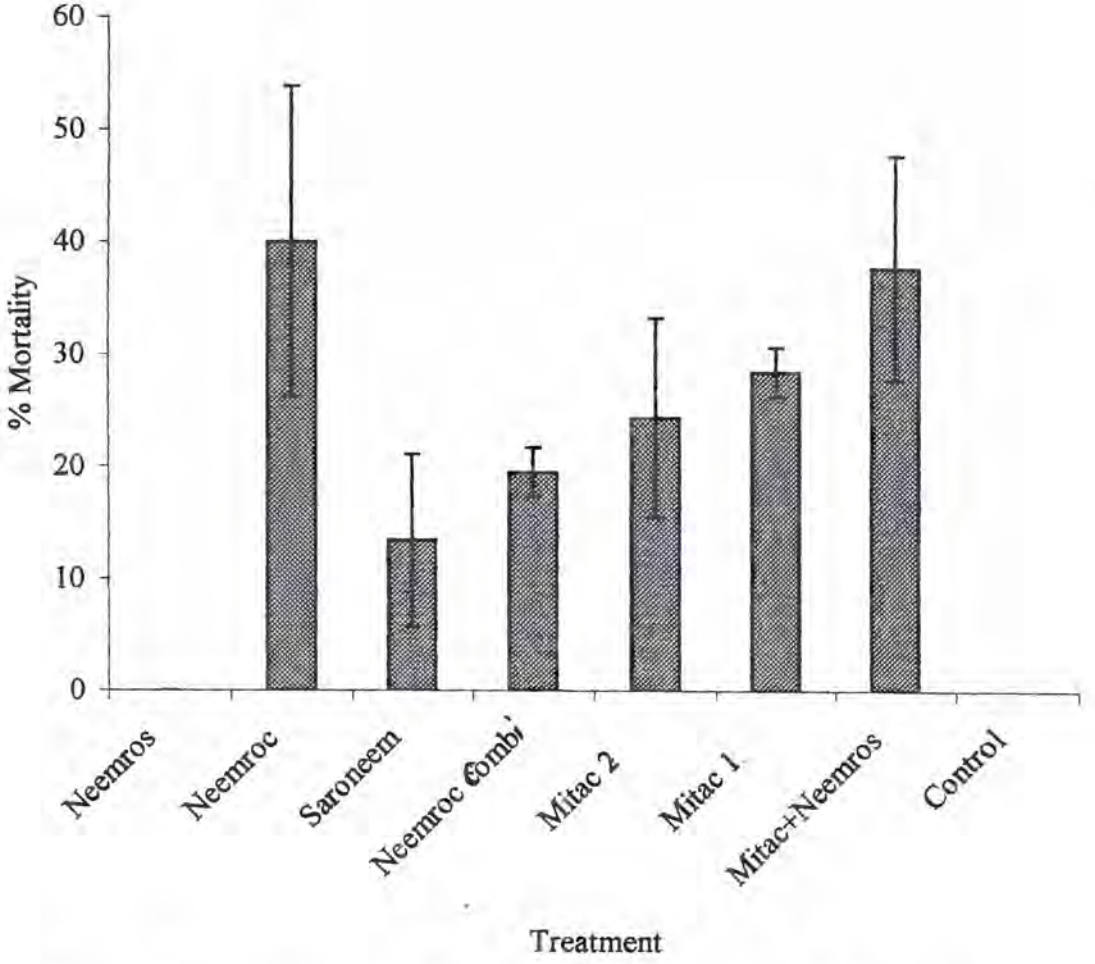


Fig. 3a. Percent mortality (Mean ± SE) of *T. Urticae* after 12 h of exposure to treatment.

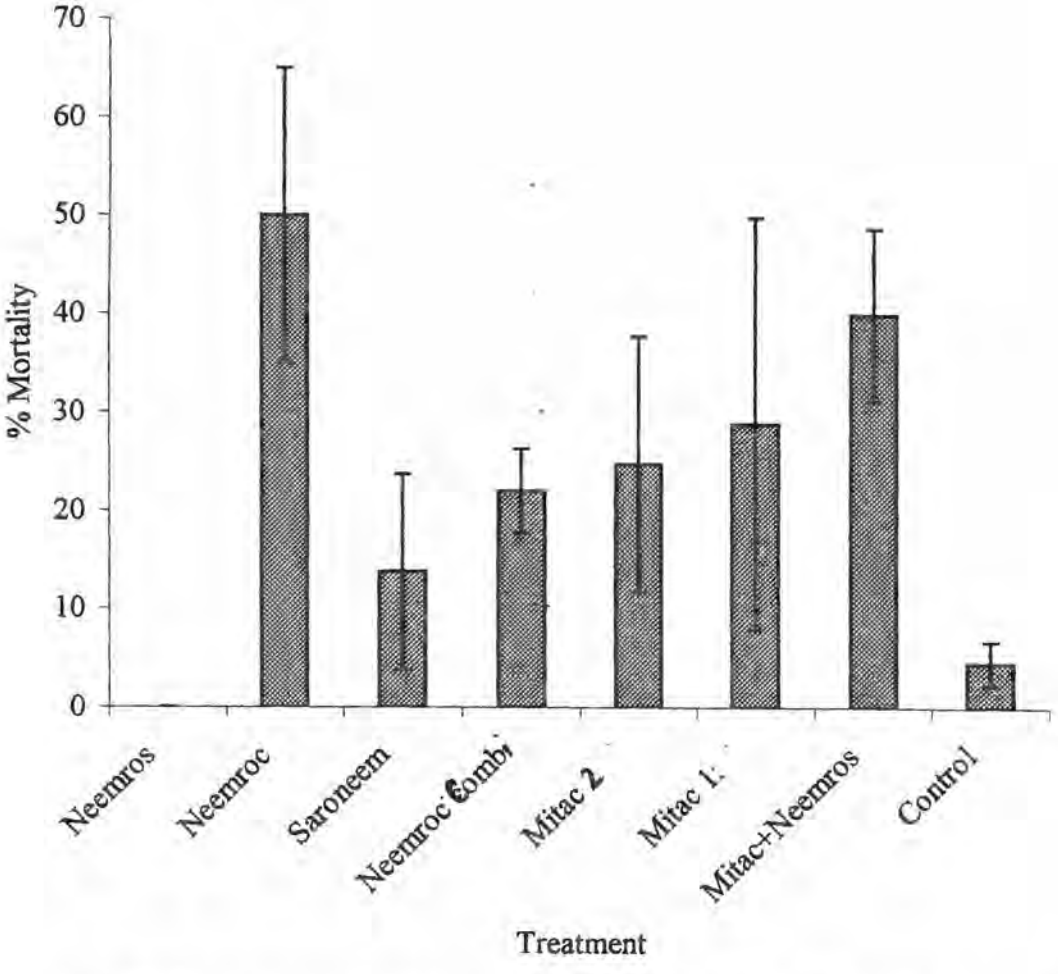


Fig 3b. Percent mortality (Mean $\pm$ SE) of *T. Urticae* after 24h of exposure to treatment.

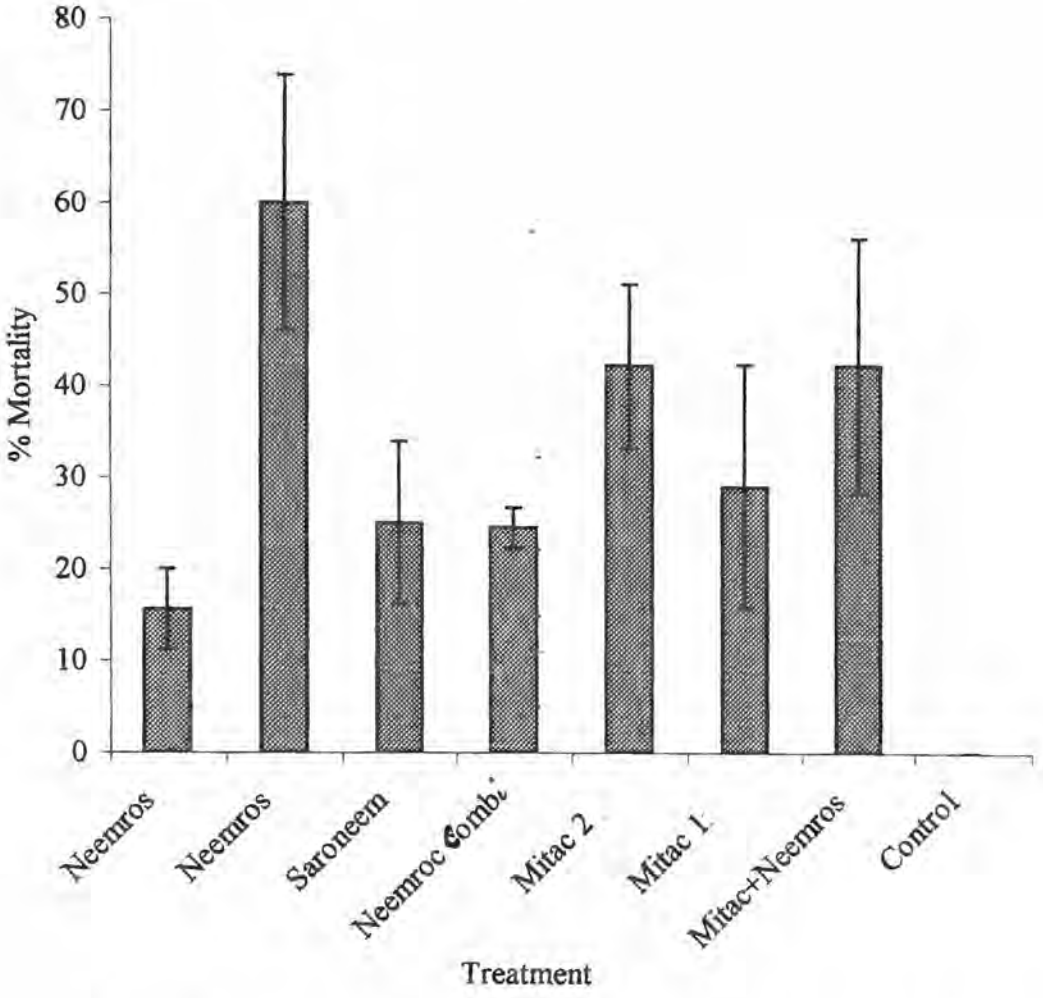


Fig. 3c. Percent mortality ( Mean ± SE ) of *T. urticae* after 48h of exposure to treatment.

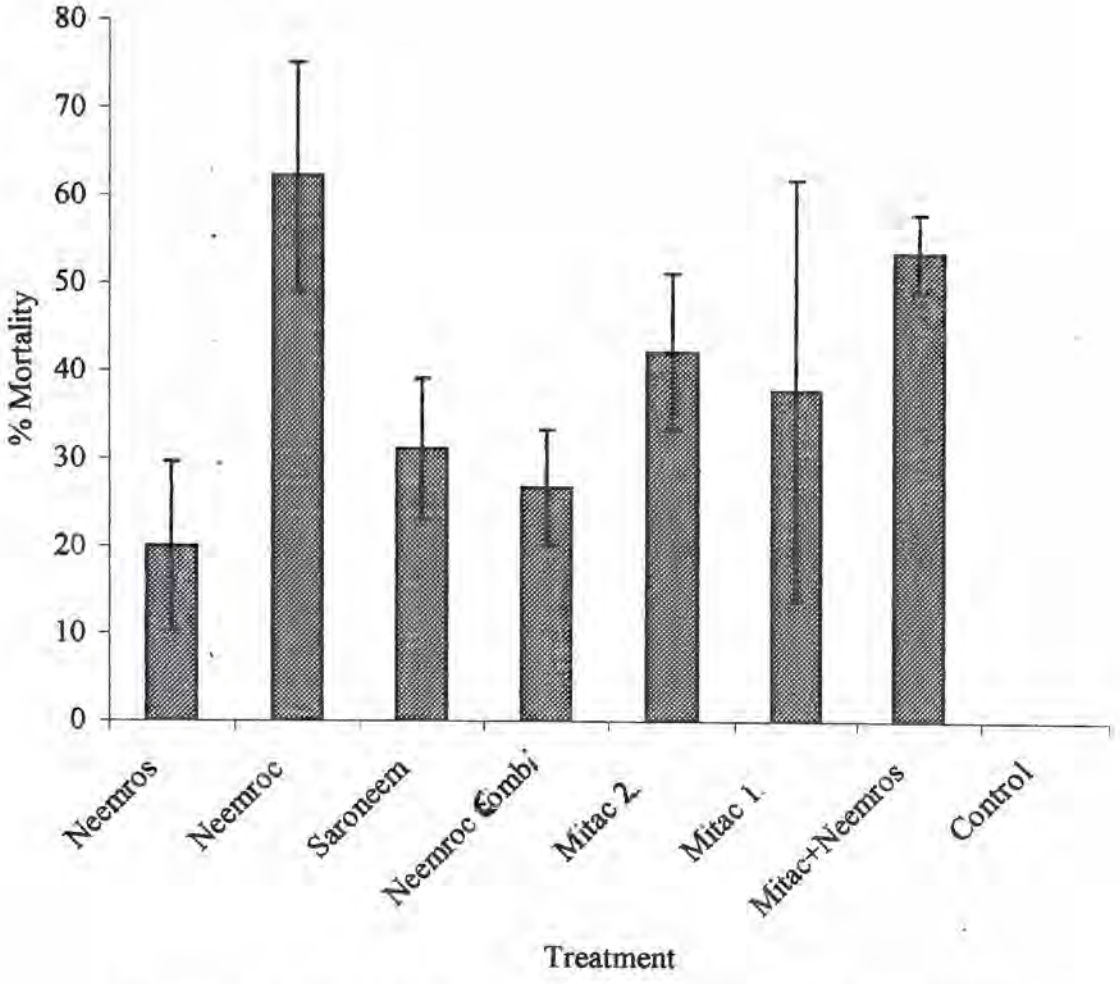


Fig. 3d. Percent mortality ( Mean ± SE) of *T. urticae* after 72h of exposure to treatment.

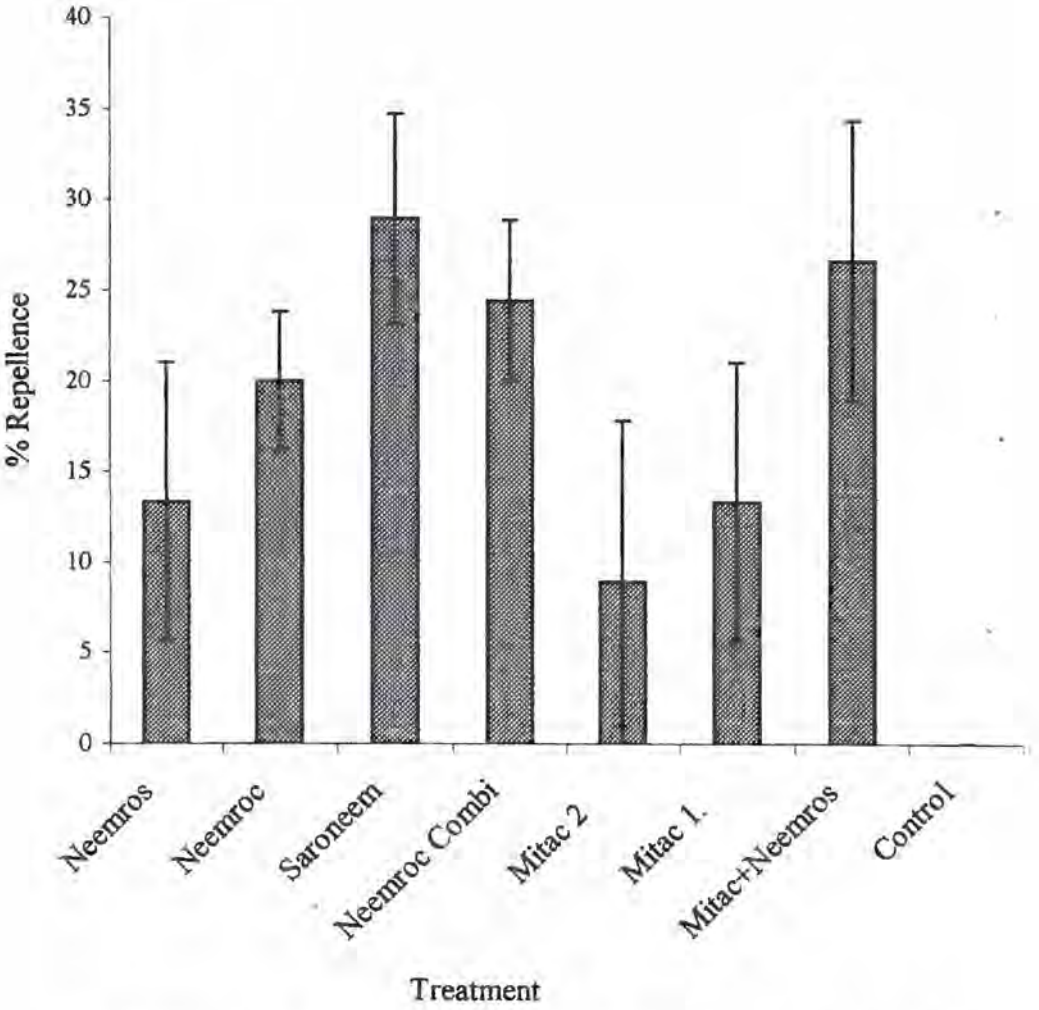


Fig. 4 a. Percent repellence (Mean ± SE) of *T. urticae* after 12h of exposure to treatment.

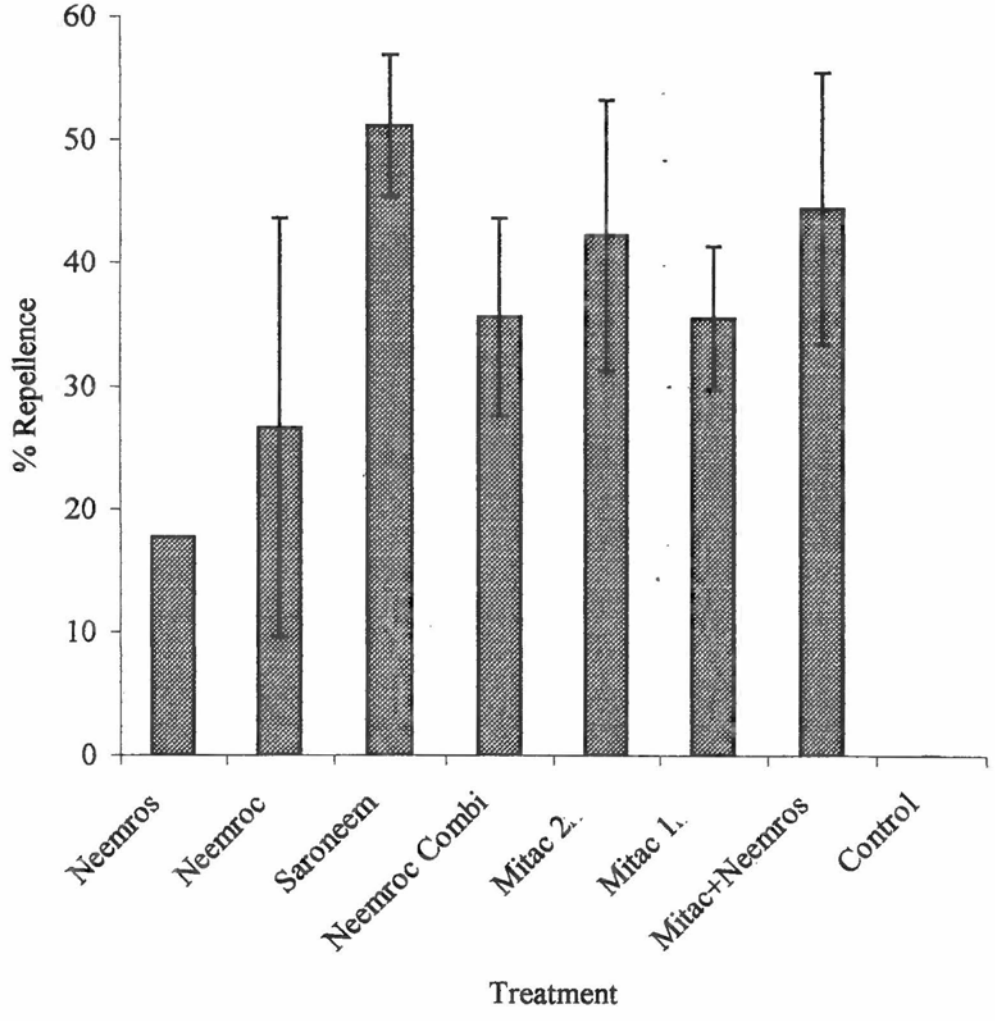


Fig. 4b. Percent repellence (Mean $\pm$ SE) of *T. urticae* after 24h of exposure to treatment.

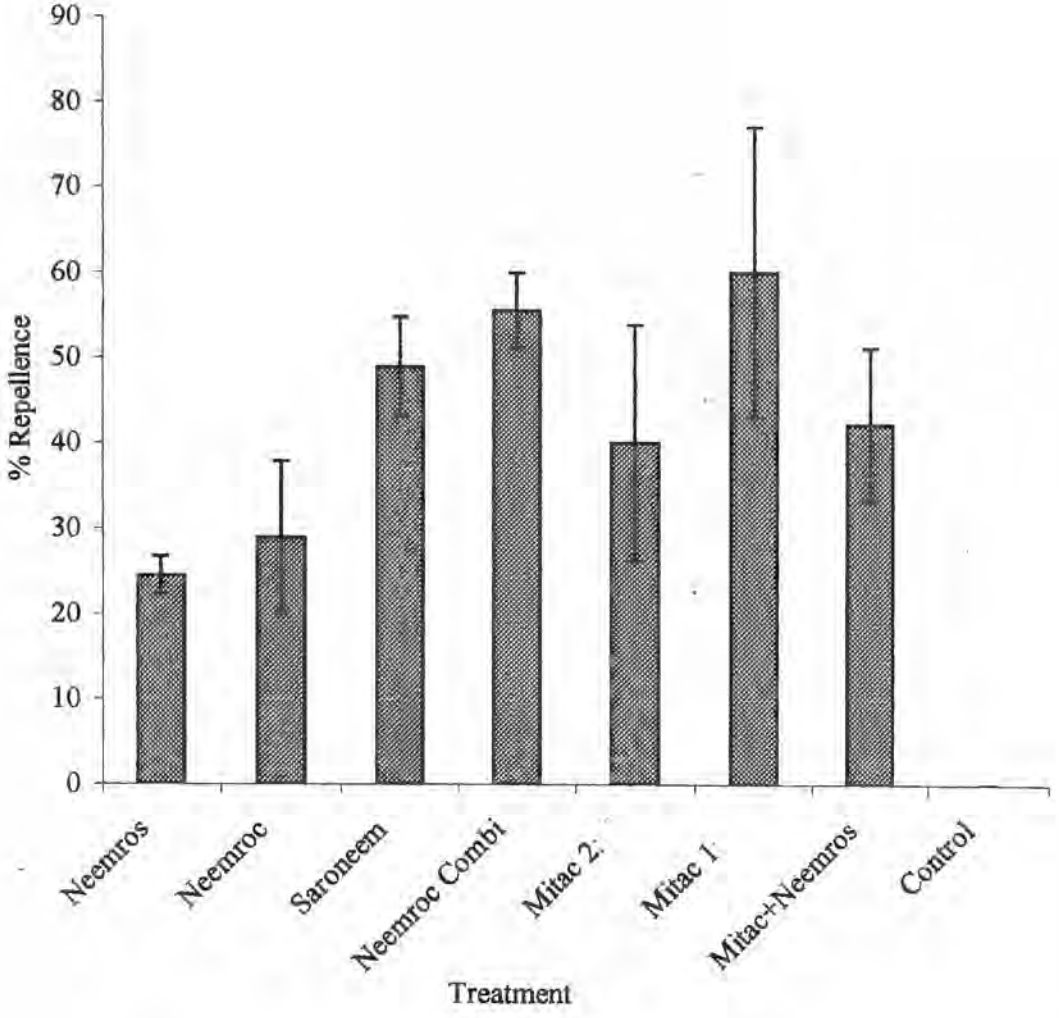


Fig.4c. Percent repellence (Mean ± SE) of *T. urticae* after 48h of exposure to treatment .

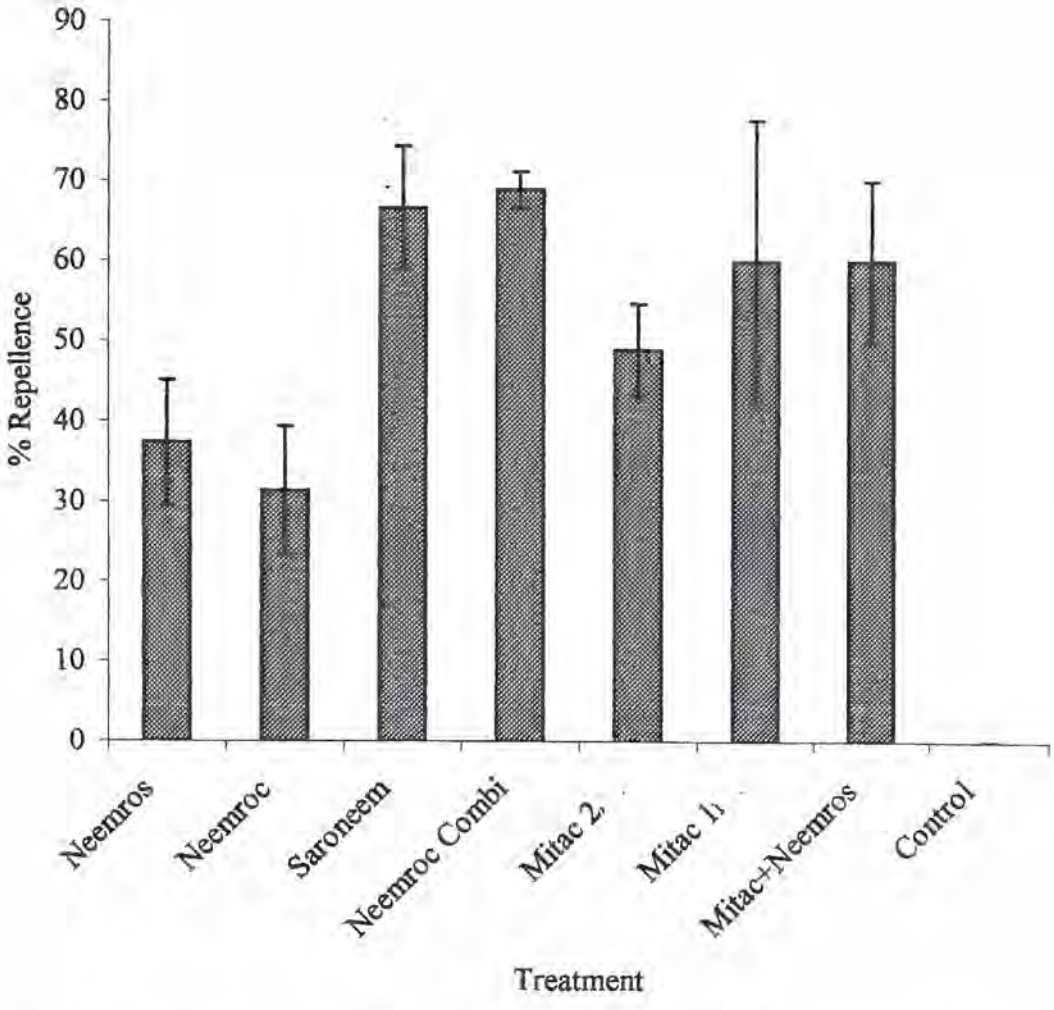


Fig. 4d. Percent repellence (Mean ± SE) of *T. urticae* aftre 72h of exposure to treatment.

Table 4 Feeding response of female *T. urticae* on tomato leaf discs. Data are averages of three replications of 15 mites each.

| | Number of mites on leaf discs (Mean ± SE) 24h | | | Number of mites on leaf discs (Mean ± SE) 72h | | |
|---|---|---|---|---|---|---|
| Treatment/dosage | Treated | Untreated | t value | Treated | Untreated | t value |
| Neemros (30 g/l) | 5 ± 0.58 | 7.7 ± 1.2 | 2.0* | 4 ± 0.58 | 9.3 ± 1.2 | 4.0* |
| Neemroc(20 ml/l) | 0.3± 0.3 | 10.7± 1.7 | 6.1* | 0 | 8.3 ± 0.9 | 9.5* |
| Saroneem(6 ml/l) | 0 | 10.3± 1.5 | 7.1* | 0.7 ± 0.3 | 10.3± 1.5 | 6.5* |
| Neemroc Combi (1 ml/l) | 1.0 ±1.0 | 8.7 ± 1.7 | 3.6* | 1.3 ± 1.3 | 10.3± 2.6 | 3.1* |
| Mitac 2 (ml/l) | 2.3± 1.5 | 12.3± 1.8 | 4.4* | 2.3 ± 1.5 | 12.3±1.5 | 4.7* |
| Mitac (1 ml/l) | 1.3± 0.9 | 11.3± 3.2 | 3.0* | 2.0 ± 1.5 | 12.0± 2.5 | 3.4* |
| Mitac+Neemros (1ml +30 g/l) | 0 | 9.0 ± 0.6 | 15** | 1.0 ± 1.0 | 9.3 ± 1.5 | 4.7* |

* Test of significance by student t – test: *P = 0.05; ** P = 0.01

Table 5: Number of eggs laid by *T. urticae* on tomato leaf discs.

| | Number of eggs (Mean ± SE) 24h | | | Number of eggs (Mean ± SE) 72h | | |
|---|---|---|---|---|---|---|
| Treatment/ dosage | Treated | Untreated | t value | Treated | Untreated | t value |
| Neemros (30 g/l) | 8.3± 2.7 | 20.3 ± 6.2 | 1.8* | 1.0± 1.0 | 23.7± 8.2 | 2.7* |
| Neemroc (20 ml/l) | 0 | 29.7 ± 6.7 | 4.4* | 34.3± 10.3 | 0 | 3.3* |
| Saroneem (6 ml/l) | 0 | 21.3 ± 8.1 | 2.6* | 0 | 30.7± 7.5 | 4.1* |
| Neemroc Comb 1 (ml/l) | 0.7± 0.7 | 19.3±8.5 | 2.2* | 5.7 ± 5.7 | 23.3 ± 3.8 | 2.6* |
| Mitac (2 ml/l) | 0.7± 0.3 | 29.0± 6.4 | 4.4* | 0 | 14.0± 4.3 | 2.8* |
| Mitac (1 ml/l) | 1.0± 1.0 | 35.3 ± 7.3 | 3.0* | 0 | 8.3± 4.4 | 3.4* |
| Mitac +Neemros (1ml +30 g/l) | 0.3± 0.3 | 13.3 ± 3.2 | 4.1* | 0 | 20.0±11.3 | 1.8* |

* Test of significance by student t – test, at P = 0.05.

### 4.1.4 Effects of Neem Formulations and Mitac on the Number Pre-imaging Stages of *T. urticae*

Table 6 Number of pre-imaging stages of *T. urticae* on tomato leaf discs treated with neem formulations and Mitac.

| | Mean eggs/days [x] | | | Mean nymphs/days [x] | | |
|---|---|---|---|---|---|---|
| Formulation/dosage | DAE[y] | | | DAE[y] | | |
| | 2 | 4 | 6 | 2 | 4 | 6 |
| Neemros (30 g/l) | 31.3b | 37.0b | 109.7b | 0.7b | 12.3ab | 26.3a |
| Neemroc (20 ml/l) | 3.3c | 3.7c | 1.0c | 0.3b | 0.3c | 0b |
| Saroneem (6 ml/l) | 12.0c | 13.7c | 8.0c | 0b | 3.3bc | 1.7b |
| Neemroc comb(1ml/l) | 11.0c | 8.0c | 3.7c | 0.3b | 4.0bc | 1.7b |
| Mitac (2 ml/l) | 3.7c | 1.7c | 2.0c | 0b | 2.3bc | 0.3b |
| Mitac (1 ml/l) | 8.3c | 5.7c | 4.7c | 0.3b | 2.3bc | 1.3b |
| Mitac + Neemros (1ml + 30 g/l) | 4.0c | 1.7c | 1.0c | 0b | 2.0bc | 0.7b |
| Control | 106a | 159a | 226.7 | 10.7a | 21.0a | 32.7a |

[x] Within columns, means followed by a common letter are not significantly different at P = 0.05 (Ryan-Einot-Gabriel-Welsch Multiple Range Test).

[y]DAE - Number of days after treatment exposure.

Table 6 shows that the duration of exposure to treated plants affected the number of pre-imarginal stages (eggs and nymphs). The results further indicate that, except for the Neemros treated leaves, there was reduced oviposition and consequently fewer nymphs on neem treated plants, similar to the standard acaricide. The mean number of eggs and nymphs on treated leaf discs varied from 1 - 14 and 0 - 4 respectively, compared to 106 - 227 eggs and 10.7 - 33 nymphs for the control. The results show that, except for Neemros, all the other neem formulations reduced the number of eggs and nymphs and consequently resulted to lower infestation of the tomato plants

The results further show that, the number of nymphs slightly increased at day 4 in Saroneem, Neemroc Combi, Mitac 2m/l, and Mitac+Neemros treated leaves. However, a gradual decrease in the number of nymphs to an average of less than 2 nymphs per leaf disc occurred at day 6 (Table 6). It was observed that the larvae actually hatched but died at an early developmental stage. The trend was different on Neemroc treated leaf discs in which the average number of nymphs remained less than 1 in days 2 and 4. No nymphs were found on leaves on day 6.

### 4.1.5 Effects of Treatments on predatory mites, *Phytoseiulus persimilis*

Table 7 shows the mortality of *P. persimilis* in different formulations of neem and the synthetic acaricide. There were significant differences ($P > 0.05$) in mortality of *P. persimilis* between treatments.

Table 7 Mortality of female *P. Persimilis* exposed to neem formulations and Mitac after 48h.

| Treatment | Concentration | [x]%mortality (Mean ± SE )[y]$_z$ |
|---|---|---|
| Neemros | 30 g/l | 13.0± 6.7 bc |
| Neemroc | 20 mls/l | 40.0 ± 0 ab |
| Saroneem | 6 mls/l | 33.33± 17.6 abc |
| Neemroc comb | 1 ml/l | 26.7± 6.7 abc |
| Mitac | 2 mls/l | 80.0± 11.5 a |
| Mitac | 1 ml/l | 73.3± 6.7 ab |
| Mitac + Neemros | 1 ml + 30 g/l | 66.7± 24.0 ab |
| Control | Untreated | 0 c |

[x] Mortality after correction using Abott (1925) formula.

[Y] Five *P. Persimilis* were used for each treatment replicated three times.

$_z$ Within the column, means followed by a common letter are not significantly different at P = 0.05 (Ryan-Einot-Gabriel-Welsch Multiple Range Test).

The toxicity of the neem formulations and acaricide in decreasing order was as follows: Mitac 2 mls/l > Mitac 1 ml/l > Mitac + Neemros > Neemroc > Neemroc comb > Saroneem > Neemros. Neem formulations were less toxic to the preda edaceous mites than to Mitac. Although Neemros (WP) was the least toxic, its toxicity increased with addition of Mitac. The mortality of *P. persimilis* on leaf treated with Mitac at a dosage of 2m/l was significantly higher than that of Mitac mixed with Neemros.

### 4.2 Efficacy of Neem Products and Mitac on *T. urticae* in Green house

The efficacy of various neem formulations and the standard acaricide, Mitac, on *T. urticae* population in the greenhouse is shown in Figure 5. Except for Neemroc and Mitac treatments, the other neem formulations did not prevent the multiplication of mites. However, in all treatments, the populations of mites were significantly lower than in the control. Compared to Mitac, the neem formulations, except Neemroc, did not provide adequate control of mite populations. The effectiveness of Neemroc was equivalent to that of Mitac.

The foliar damage by *T. urticae* corresponds to the level of control of mites. It was generally higher on neem treated plants. However, Neemroc was comparatively more effective in reducing foliar damage by mites. Neemroc and Mitac treatments were equally effective in reducing foliar damage (Table 8). The neem formulations reduced foliar damage to different levels, which, with

the exception of Neemros, Saroneem and Neemroc Combi, were significantly lower than the control.

The results also indicate that Mitac and Neemroc had longer residual effects than the other formulations. This is shown by lower feeding damage indices and gradual decrease of number of mites at each spraying date (Fig. 5).

**Defoliation and Leaf curl**

Plants treated with neem formulations had more defoliation, flowers and immature fruits drop than Mitac treated plants. The highest defoliation and immature fruits drop was observed on untreated plants. Neemros, Saroneem and Neemroc combi treated plants showed signs of leaf curly due to mite damage. These symptoms were not observed in plants treated with Neemroc and Mitac.

**Fruits yield**

There were significant variations in number of fruits in plants treated with different neem formulations (Table 8). Neemroc treated and untreated control plants without mites had significantly higher number of fruits than in other treatments. The untreated plants had significantly lower number of fruits relative to treated plants.

The yield of tomato (by weight) according to treatment were in the following

order: Untreated without mites > Neemroc > Mitac 2ml/l > Mitac + Neemros > Mitac 1ml/ > Neemros > Saroneem > Neemroc combi > Untreated control with mites (Table 8).

### 4.3 Plant Diseases

All plants developed powdery mildew (*Sphoerotheca fuliginea*) symptoms at the 8$^{th}$ week after transplanting. The neem formulations also appeared to affect infection by powdery mildew. However, plants treated with mitac and the untreated control, with and without mites, were more susceptible than the neem treated plants. Also one plant showed black-end disease symptoms. These disease, however, did not seem to have noticeable effect on yield of tomatoes.

Table 8 Effects of repeated application of neem and Mitac formulations on population of *T. urticae* damage and yield of tomatoes in the green house.

| Treatments/Dosages | Number of Mites | Feeding damage index * | Number of fruits[x] | Fruit weight weight [y] (g) |
|---|---|---|---|---|
| Neemros (30g/l) | 624±55.6 b[z] | 2.3±0.30 a | 22±2.0 abc | 57.8±3.9bcd |
| Neemroc (20m/l) | 142.3±27.3 c | 0.9±0.05 b | 31.3±2.6 a | 70.9±2.5 b |
| Saroneem (6ml/l) | 655.5±56.2 b | 2.6±0.30a | 26±3.0 abc | 52.9±3.9 cd |
| Neemroc combi (1ml/l) | 633.5±56.4 b | 2.6±0.30a | 24±0.3 abc | 47.2±3.8 dc |
| Mitac (2ml/l) | 58.0±27.6 c | 1.0±0.05 b | 25±2.1 abc | 65.1±3.9 bc |
| Mitac (1ml/l) | 90.7±29.2 c | 1.2±0.08 b | 23±1.2 abc | 62.1±3.0 bc |
| Mitac + Neemros (1ml+20g/l) | 94.9±27.9 c | 1.2±0.10 b | 23.3±0.3abc | 63.2±3.0 bc |
| Untreated (with mites) | 846.3±93.0 a | 2.7±0.30a | 18.3±2.3 c | 38.4±3.8 e |
| Untreated (without mites) | - | - | 29.3±1.5 ab | 83.8±3.1 a |

*Damage rating (LDI):0 = No damage, 1= Few mites attack with small patches, 2= Large feeding patches < 25% Leaf area, 3= Feeding patches > 25% leaf area, 4= Entire leaf with feeding marks but still green, 5= Necrotic and chlorotic area, the leaf begins to shrivel.

[x] Sum of fruit numbers per plant.

[y] Sum of fruit weights per plant.

[z] Within column, means followed by common letter are not significantly different at P = 0.05, according to Ryan-Einot-Gabriel-Welsch Multiple Range Test.

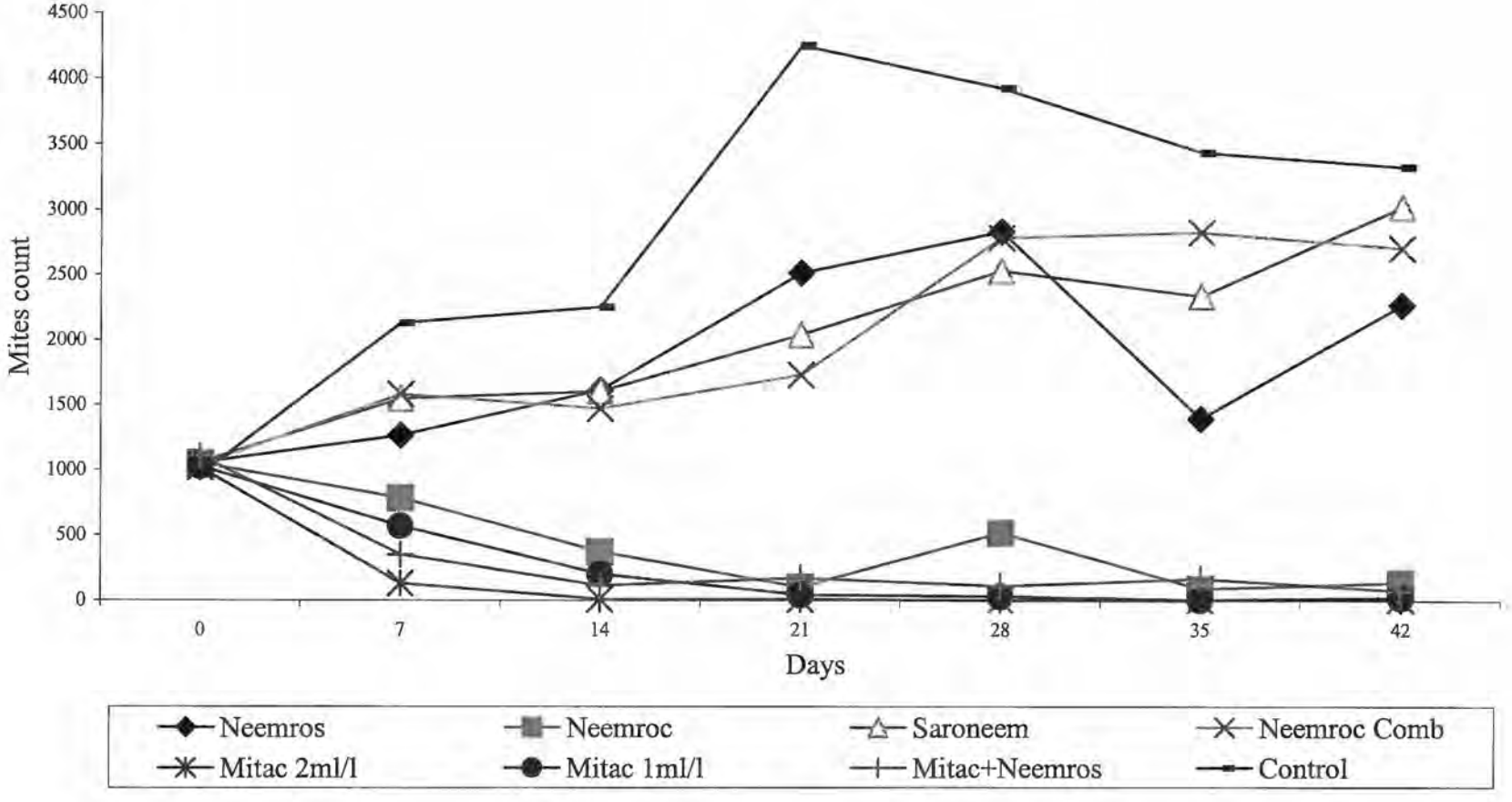


Fig. 5. The effect of repeated sprays of different formulations of neem and Mitac on the population (number of mites) of *T. urticae,* in the green house.

# CHAPTER FIVE

## 5.0 DISCUSSION

### 5.1 Laboratory Evaluation

Tomatoes are cultivated in many developing countries. However, to get a satisfactory crop yield, huge quantities of synthetic pesticides must be applied. A reduction of synthetic chemical input is highly desirable, not only for economic, but also for ecological reasons. Successfully tested pesticides of plant origin, such as neem extracts, could help subsistence farmers to improve crop yields, with a minimal cash input in the first place because they might be cheaper and also they are environmentally friendly (Schmutterrer 1995). Substitution of synthetic pesticides with neem extracts might, however, be feasible only to a certain degree.

Neem extracts have been reported to have different components with insect pest antifeeding and antioviposition properties (Steets, 1976; Uebel *et al.*, 1978; Isman *et al.*, 1991). The effects of neem extracts on feeding and oviposition deterrence of *T. urticae* have also been reported (Schauer and Schmutterer, 1981). The main compounds in neem which have bio activity belong to a general class of natural products called "teriterpenes" or limonoids. So far at least nine limonoids have demonstrated ability to block insect growth, affecting a range of species. Azadirachtin, salannin, meliantriol and nimbin have been reported as most significant limonoids (BOSTID, 1992).

Azadirachtin is the main active ingredient of neem extracts (Schmutterrer and Ascher, 1984). It appears to cause 90 percent of the effects on most pests. It does not kill insects immediately, but, instead, it both repels and disrupts their growth and reproduction. The rest of the named limonoids are reported to inhibit feeding but do not influence insect molting (Steets, 1976; BOSTID, 1992). There are minor neem ingredients, for example deacetylazadirachtinol (BOSTID, 1992), which paralyze the "swallowing mechanism" of insects and therefore prevent feeding.

In the current study, the performance of neem products against mites was not as effective as that of the synthetic acaricide, except for Neemroc EC. Jaipal *et al.*, (1984) reported that considerably higher doses of neem extracts are required for pest control. The current study has indicated poor efficacies of Neemros powder (0.5% azadirachtin) against mites in the laboratory even when the dosage applied was increased.

The recommended application rate of 25 g/l of Neemros is reported to be effective against a wide range of insect pests including leaf miner (*L. trifolii)*, diamond back moth (*P. xylostella), C. binotalis,* and *H. undalis* (Sanguangpong and Schmutterer, 1992). No activity was found against *T. cannabarinus* on eggplants (Fagoone, 1987).

The LC50 (38g/l) established in the current study was higher than the recommended dosage. The poor performance of Neemros powder is further shown by the fact that, even at higher dosages than the recommended application rate, mortality, repellence, and number of pre-imarginal stages was not stastically different from the control. These findings clearly show that Neemros is not particularly effective for control of two spotted spider mites inspite of its reported effectiveness against several insect pests.(Schmutterer, 1995; Hellpap and Dreyer, 1995; Mansour and Asher, 1984).

Hellpap and Dreyer (1995) recommended a dosage of 15 - 30g/l of Neemros for control of low incidence of pests or highly susceptible pests, and 40 - 60 g/l for high incidence of pests or control of moderately susceptible pests. These latter dosages are higher than the $LC_{50}$ reported in this study, indicating that some pests require a higher dosage than that recommended. There are no recommendations for the application of neem formulations to control two spotted spider mites.

Azadirachtin, the main active ingredient in neem powder is reported to have less solubility in aqueous extracts (Mansour and Ascher 1983, 1984). It has been reported that azadiradirachtin contributes nothing to the toxicity of neem product against two spotted spider mites (Mansour and Ascher, 1983; Singuanpong and Schmutterer, 1992; Schmutterer, 1995).

These observations are consistent with the observations reported by Mansour and Ascher (1984) that neem seed kernel extracts prepared using various solvents affected the behaviour of *T. cannabarius* where as, the extract prepared using water was inactive. Extracts from lipophilic solvents were reasonably acaricidal (Sanguanpong and Schmutterer, 1992). These authors also reported that, the addition of azaditachtin to any of the petrol ether or methanol phases of the two extracts did not improve efficacy, and that neem azadirachtin was practically inactive. These findings are confirmed by the results in the current study in which Neemros, whose main active ingredient is azadirachtin, was not effective against the mites.

Among the neem formulations, Neemroc EC provided better protection against mites than all the others. It also provided equivalent or protection than Mitac. The potency and insecticidal properties of the active principal in neem is represented by tertanortriterpenoid, mostly azadirachtin (BOSTID, 1992) but the acaricidal activity of azadirachtin is very poor (Singuangpong and Schmutterer, 1992).

Neemroc EC has smaller amounts of azadirachtin (0.03%) than all neem formulations, and yet, had good activity on mites than Neemros (0.5%), Saroneem (1%), and Neemroc comb (0.5%). The effectiveness of Neemroc was

probably enhanced by the oil emulsion rather than its azadirachtin content (Schmutterer, 1995). The blocking of the stigma caused by the oil film spreading on the body of mites is probably the cause of death (Sirvastava and Parma, 1985; Chinaella and Rovesti, 1992). This probably explains the good activity of Neemroc even at the low concentrations, and therefore, shows the importance of the type of formulation in relation to control of the pest.

All neem formulations were good feeding and oviposition deterrents, except Neemros. Neemroc had excellent mite feeding and oviposition deterrence effect. These findings concur with those of Chinaella and Rovesti (1992), who reported that adult mites moved to untreated plants of soybeans, an indication of repellence or antifeedant activity. The antifeedant effects of neem products have been reported elsewhere. For example, Jacobson *et al.*,(1978), Schauer and Schmutterer (1981) and Dimetry and Schmidt (1992), reported that neem oil, and some pure compounds and formulations from the neem tree elicited a very good feeding deterrence activity to citrus red mites *(P. citri)*, the two spotted spider mites *T. urticae* and bean aphids (*A. fabae*).

Similar antifeedant effects have been reported by Karel (1986) on *Ootheca bennigseni*, and were attributed to the triterpenoids, azadirachtin and salannin. The current study has also confirmed that with the exception of aqueous

extracts, neem formulations (Neemroc, Saroneem, and Neemroc comb) can deter feeding of two spotted spider mites.

Neemroc was effective in reducing the number of preimarginal stages of mites. In this study it was observed that Neemroc has some ovicidal effect because the number of nymphs was significantly lower compared to the control. Some of the eggs failed to hatch and for those, which hatched, the larvae died, probably due to starvation. This could have been a result of the antifeedant effect of the formulation. Chianella and Rovesti (1992) made similar observations. It has also been suggested that this effect be due to the presence of compounds acting as ovicides and adulticides (Chianella and Rovesti, 1992).

For practical pest control, even simple alcohol macerates prepared from different parts of the neem tree have proved to be effective against a wide range of pests (Hellpap and Dreyer, 1995). Alcohol extraction is reported as the most direct process for producing neem based pesticidal material in concentrated form because neem limonoids are highly soluble in alcohol solvents and less soluble in water (BOSTID, 1992). According to BOSTID (1992), alcohol extracts are 50% more concentrated than water extracts.

Saroneem, an isopropyl alcohol extract had a high azadirachtin content (1% azadirachtin) than the rest of neem formulations. Saroneem showed a

remarkable increase in mortality of two spotted spider mites as compared to the use of Neemros powder and Neemroc Combi, but the effects were less than those of Neemroc and Mitac. The formulation had strong repellence effect as the dosage increased. These observations are consistent with the findings of Dimetry *et al* (1993), in which Morgosan – 0 (a commercial alcohol extract of neem) had a pronounced deterrent effect on two spotted spider mites.

Ovipositional deterrence due to neem limonoid results into a decrease in the number of preimaginal stages. The number of eggs and nymphs was lower in Saroneem treatment, but not as low as that of Mitac treatments. Mansour and Ascher (1983, 1984) found that when 24h - old *T. urticae* eggs were sprayed with the methanolic neem seed kernel extracts solution, not only was post embryonic development retarded considerably, but mortality also set in progressively. Dimetry *et al.*, (1993) also found that the hatchability of *T. urticae* eggs was reduced by alcohol extracts.

The current study shows that the enriched extract in Neemroc combi (0.5% azadirachtin) had low toxicity to two-spotted spider mites compared to that of Saroneem, Neemroc and Mitac. Lidert (1990) reported that low toxicity of the enriched extracts was due to their low oil contents. The low mortality of mites caused by Neemroc Combi at higher dosages was probably due to low solubility of limonoids, as well as low oil contents.

The activity of Neemroc combi was, however, higher than that of Neemros WP. In general, enriched extracts are more effective than aqueous extracts (Lidert, 1990). Probably the small oil content in enriched extract had some effects on mortality of mites (Lidert, 1990). The dosages applied seemed to be very low to have good effect on mites. Lidert (1990) also reported that, azadirachtin in enriched extracts contributes to insect repellence but higher concentrations are required for high mortality to be achieved.

Neemroc combi also reduced the number of eggs laid considerably as compared to the control and to Neemros. However, the number of eggs oviposited increased with time of exposure. This shows that, the residual effects of Neemroc combi were reduced with time. Although Saroneem and Neemroc Combi also led to a remarkable reduction of infestation by mites, it was less than that caused by Neemroc and Mitac. This indicates that, the use of Saroneem and Neemroc combi cannot provide total protection to the crop.

It is also evident that a combination of Mitac and Neemros increased effectiveness, which was better than their separate efficacy. This is shown by increased mortality of mites, repellence and reduced feeding and oviposition. This suggests that a mixture of the two has synergistic effect. BOSTID (1992) reported that a mixture of neem extract and a synthetic pesticide can add a rapid " knock down" to neem's ability to suppress the subsequent rebound in the pest

population. Sirvastava and Parmer (1985) reported a synergistic effect when *A. Indica* and Malathion were mixed and used against *Tribolium castaneum*.

### 5.1.1 Effect of neem formulations on predatory mites

This study has shown that the synthetic acaricide, Mitac, kills the natural enemies more effectively than neem products. Several other studies (Mansour *et al.*, 1986; Schmutterer, 1992 and Meyer, 1996) have also reported that neem formulations were less toxic to predatory mites.

Sirvastava and Parmer (1985) found that there are differences in the structure of the stigma of the citrus red mite and predatory mites. The stigmas of the citrus red mites are much more easily blocked by the oil formulation than those of the predatory mites. This is probably the reason for good effectiveness of Neemroc against *T.urticae* but was less active against predatory mites.

### 5.1.2 Phytotoxicity

Neemroc was phytotoxic at higher dosages. Similar observations were reported by Chianella and Rovesti (1992). The observed symptoms were mainly the detachment of the epidermis, leaf burning and blockage of vegetative growth. Schmuterrer (1995) also reported that neem oil was phytotoxic. Other neem treatments applied in this study did not show phytotoxicity on the tomato plant leaves.

### 5.1.3 Plant Disease

Powdery mildew constitutes a major problem in cucurbits, tomato and ornamental plants. Neem formulations appeared to prevent fungal infestation more effectively than Mitac. Similar observations have been made elsewhere. For example, Kleeberg (1992) observed that neem extracts prevented powdery mildew in soybeans. Powdery mildew generally, is reported to be sensitive to neem seed oil applied either as a protectant or curative (Chinaella and Rovesti, 1990; Locke, 1990; Rovesti *et al.*, 1992).

## 5.2 The Efficacy of Neem formulations in the Greenhouse

The effectiveness of neem oil emulsion (Neemroc) against mites was confirmed in greenhouse findings. The formulation provided better protection against two-spotted spider mites, showed by less number of mites and lower feeding damage compared to the conventional acaricide at the recommended concentration of 20 ml/l. The better protection over other neem formulation is attributed mainly to the oil content (Chinaella and Rovesti, 1992). Other neem formulations (Saroneem and Neemroc combi) showed significantly less protection.

In this study there were significant differences between plant treated by neem formulations (with the exception of Neemroc, which had the same LDI as Mitac) and the plants treated with Mitac. The LDI in this study ranged from 0.9 to 2.7. Nihoul *et al.*, (1991) reported that only scores of 0.1 to 3.0 are

considered, because damage level above this is not acceptable in a crop.

The highest leaf damage was observed in the control with mites (LDI=2.7), Saroneem (LDI=2.6) and Neemroc combi (LDI=2.6). Plants treated with these formulations showed about 50% leaf defoliation and small sized fruits with significantly less fruit weight compared to the other treatments. The plant damage index described here was based on the upper part of the tomato plant leaves (10 apical leaves). Stacey (1983) found that of all the leaves on a plant, the top 12 leaves contribute most to the yield. The same author found that the removal of about 25% of the photosynthetic area did not affect yield, and that loss of 50% of the foliage reduces yield by 16%. Hussey and Scope (1985) fixed the critical threshold at mean LDI of 2.0 which corresponds, on their scale, to a reduction of foliage by 30%. Damage to leaves near truss of setting fruit is likely to cause yield loss at a rate approximately equal to the proportion of leaf area affected.

In this study *T.urticae* was present practically on all the ten upper leaves. Except for Neemroc treated plants, all other plants treated with neem formulations scored LDI above 2, while Neemroc and Mitac treated plants had LDI of less than 1.2. According to Nihoul *et al.*, (1991) yield losses can be expected when mean leaf damage index reached 2.0 – 2.5.

Mites effects on tomato yield were less significant, most probably due to the time of infestation. Mites infestation was done at flower initiation stage. Stacey (1983) commented that there is a delay of 5-6 weeks between the on set of damage and diminished – yield response. Klopczynska and Tomeczyk (1986) concluded that most severe mite damage occurs early in the vegetative period. When the infestation increased after flowering, no influence on yield resulted.

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATION

This study suggests that there is potential for using neem formulations to control two-spotted spider mites in tomatoes. Neemroc EC treatment provided better protection almost like that of Mitac treatments. While Saroneem and Neemroc combi provided moderate protection. Neemros had the least protection. Neemroc EC formulation had feeding and oviposition deterrence as well as causing mortality of two-spotted spider mites. Saroneem and Neemroc combi had repellence, feeding and oviposition deterrence and caused little mortality. A mixture of Mitac and Neemros also gave better protection than that of Mitac and Neemros separately, most certainly a result of synergistic action.

Neemros WP did not succeed in controlling or reducing the level of infestation on tomato leaves both under laboratory and greenhouse conditions. Therefore, the treatment is not recommended for the control of *T. urticae*. Neemroc EC at the rate recommended by the manufacturer is considered, from the results of this study, an alternative to the synthetic acaricide.

Saroneem and Neemroc combi gave some protection against two spotted spider mites by causing feeding inhibition and repellence more than mortality. Application of neem formulations especially Saroneem and Neemroc combi can reduce mites infestation to a certain degree but cannot provide complete

protection. The application of these two formulations at higher rates will provide better protection.A mixture of Neemros and Mitac was less toxic to predatory mites than Mitac alone. Therefore it is recommended to use a mixture of the two rather than Mitac alone because of lower toxicity on predatory mites. It is recommended that these trials be further tested in the field to confirm the efficacy of neem formulations under different field conditions.

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## LIST OF APPENDICES

Appendix 1a Analysis of variance of mortality and repellence data for mites exposed to Neemros treatment.

(i) Mortality:

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 0.60742547 | 0.15185637 | 7.49 | 0.0047 |
| Error | 10 | 0.20286354 | 0.02028635 | | |
| Corrected total | 14 | 0.81028901 | | | |

(ii) Repellence:

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 0.35645606 | 0.08911402 | 8.55 | 0.0029 |
| Error | 10 | 0.10425009 | 0.01042501 | | |
| Corrected total | 14 | 0.46070615 | | | |

Appendix 1b Analysis of variance of mortality and repellence data for mites exposed to Neemroc treatment.

(i) Mortality

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 4.18133436 | 1.04533359 | 17.20 | 0.0002 |
| Error | 10 | 0.60785605 | 0.0607850 | | |
| Corrected total | 14 | 4.78919041 | | | |

(ii) Repellence

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 0.13610570 | 0.03402642 | 3.75 | 0.0411 |
| Error | 10 | 0.09082804 | 0.00908280 | | |
| Corrected total | 14 | 0.22693374 | | | |

Appendix 1c Analysis of variance of mortality and repellence data for mites exposed to Saroneem treatment.

(i)Mortality:

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 1.09912618 | 0.27478154 | 11.60 | 0.0009 |
| Error | 10 | 0.23693412 | 0.02369341 | | |
| Corrected total | 14 | 1.33606030 | | | |

(ii) Repellence

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 0.25469627 | 0.06367407 | 4.99 | 0.0180 |
| Error | 10 | 0.12766052 | 0.01276605 | | |
| Corrected total | 14 | 0.38235679 | | | |

Appendix 1d. Analysis of variance of mortality and repellence data for mites exposed to Neemroc Combi treatment.

(i) Mortality:

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 0.87777914 | 0.21944478 | 3.67 | 0.0434 |
| Error | 10 | 0.59783062 | 0.059783062 | | |
| Corrected total | 14 | 1.47560976 | | | |

(ii) Repellence

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 0.13577055 | 0.03394264 | 4.48 | 0.0249 |
| Error | 10 | 0.07584082 | 0.00758408 | | |
| Corrected total | 14 | 0.21161137 | | | |

Appendix 1e Analysis of variance of mortality and repellence data for mites exposed to Mitc treatment.

(i) Mortality:

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 3.33243434 | 0.83310858 | 12.07 | 0.0008 |
| Error | 10 | 0.69002051 | 0.06900205 | | |
| Corrected total | 14 | 4.02245485 | | | |

(ii) Repellence

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 0.21562808 | 0.05390702 | 9.82 | 0.0017 |
| Error | 10 | 0.05489898 | 0.00548990 | | |
| Corrected total | 14 | 0.27052706 | | | |

Appendix 1f Analysis of variance of mortality and repellence data for mites exposed to combination of Mitac and Neemros treatments.

(i) Mortality:

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 3.47890890 | 0.86972723 | 14.27 | 0.0004 |
| Error | 10 | 0.60943450 | 0.06094345 | | |
| Corrected total | 14 | 4.08834340 | | | |

(ii) Repellence

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Conc | 4 | 0.08398001 | 0.02099500 | 2.32 | 0.1278 |
| Error | 10 | 0.09047077 | 0.00904708 | | |
| Corrected total | 14 | 0.17445078 | | | |

Appendix 2a Analysis of variance of mortality and repellence data for mites exposed to Neem formulations and Mitac treatments after.

(i) Mortality

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 0.81574424 | 0.11653489 | 5.87 | 0.0017 |
| Error | 16 | 0.31746258 | 0.01984141 | | |
| Corrected total | 23 | 1.13320682 | | | |

(ii) Repellence

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 0.21175864 | 0.03025123 | 2.36 | 0.0739 |
| Error | 16 | 0.20547676 | 0.01284230 | | |
| Corrected total | 23 | 0.41723540 | | | |

Appendix 2b Analysis of variance of mortality and repellence data for mites exposed to Neem formulations and Mitac treatments after 24h.

(i) Mortality

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 0.77863998 | 0.11123428 | 2.31 | 0.0780 |
| Error | 16 | 0.76897384 | 0.04806086 | | |
| Corrected total | 23 | 1.54761382 | | | |

(ii) Repellence

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 0.63727803 | 0.09103972 | 2.89 | 0.0373 |
| Error | 16 | 7.47809615 | 0.46738101 | | |
| Corrected total | 23 | 1.14110401 | | | |

Appendix 2c Analysis of variance of mortality and repellence data for mites exposed to Neem formulations and Mitac treatments after 48h.

(i) Mortality

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 0.871202335 | 0.12445748 | 3.25 | 0.0240 |
| Error | 16 | 0.611191532 | 0.03824471 | | |
| Corrected total | 23 | 1.48311768 | | | |

(ii) Repellence

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 0.97731707 | 0.13961672 | 3.68 | 0.0146 |
| Error | 16 | 0.60644612 | 0.03790288 | | |
| Corrected total | 23 | 1.58376319 | | | |

Appendix 2d Analysis of variance of mortality and repellence data for mites exposed to Neem formulations and Mitac treatments after 72h.

(i) Mortality

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 0.89708590 | 0.12815513 | 2.10 | 0.1034 |
| Error | 16 | 0.97509729 | 0.06094358 | | |
| Corrected total | 23 | 1.87218319 | | | |

(ii) Repellence

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 1.43266890 | 0.20466699 | | |
| Error | 16 | 0.59086222 | 0.03692889 | | |
| Corrected total | 23 | 2.02353112 | | | |

Appendix 3a Analysis of variance of data for mites pre imarginal stages exposed to Neem formulations and Mitac treatments after 2 days (Transformed Data).

(i) Eggs

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 2567.29167 | 366.76131 | 16.27 | 0.0001 |
| Error | 16 | 70.266667 | 4.39166 | | |
| Corrected total | 23 | 2637.595833 | | | |

(ii) Nymphs

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 24.06024303 | 3.43717758 | 11.48 | 0.0001 |
| Error | 16 | 4.78847264 | 0.29927954 | | |
| Corrected total | 23 | 28.84871566 | | | |

Appendix 3b Analysis of variance of data for mites pre imarginal stages of mites exposed to Neem formulations and Mitac treatments after 4 days.

(i) Eggs

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 6096.0625 | 870.86607 | 11.12 | 0.0001 |
| Error | 16 | 125.3333 | 7.83333 | | |
| Corrected total | 23 | 6221.3958 | | | |

(ii) Nymphs

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 38.2783 | 5.4683 | 8.21 | 0.0003 |
| Error | 16 | 10.6549 | 0.6659 | | |
| Corrected total | 23 | 48.9333 | | | |

Appendix 3c Analysis of variance of data for pre imarginal stages of mites exposed to Neem formulations and Mitac treatments after 6 days.

(i) Eggs

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 4760.8388 | 680.1194 | 6.32 | 0.0001 |
| Error | 16 | 172.0666 | 10.7541 | | |
| Corrected total | 23 | 4818.1667 | | | |

(ii) Nymphs

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 100.3420 | 14.3345 | 23.20 | 0.0001 |
| Error | 16 | 9.8879 | 0.6179 | | |
| Corrected total | 23 | 110.2301 | | | |

Appendix 4 Analysis of variance of mortality data for predatory mites *P. Persimilis* exposed to neem formulations and mitac.

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 3.10649867 | 0.44378556 | 4.15 | 0.0088 |
| Error | 16 | 1.71290254 | 0.10705641 | | |
| Corrected total | 23 | 4.81940121 | | | |

Appendix 5 Analysis of variance of data for mites population exposed to repeated application of neem formulations and mitac in the greenhouse.

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 14190.9789 | 2027.2827 | 56.87 | 0.0001 |
| Error | 160 | 5703.6555 | 35.6478 | | |
| Corrected total | 167 | 19894. 6343 | | | |

Appendix 6 Analysis of variance of data for leaf damage index LDI on tomatoes after repeated application of neem formulation and Mitac on two-spotted spider mites population in the greenhouse.

| Source | DF | SS | MS | F Value | P |
|---|---|---|---|---|---|
| Treatment | 7 | 11.5545261 | 1.65066466 | 13.95 | 0.0001 |
| Error | 160 | 18.9336982 | 0.1183356 | | |
| Corrected total | 167 | 30.4882243 | | | |

Appendix 7 Analysis of variance of data for tomato yield (fruits number) exposed to repeated application of neem formulations and mitac on two-spotted spider mites population in the greenhouse.

| Source | DF | SS | MS | F Value | Pr |
|---|---|---|---|---|---|
| Treatment | 8 | 384.518519 | 48.064815 | 4.28 | 0.0050 |
| Error | 18 | 202.000000 | 11.222222 | | |
| Corrected total | 26 | 586.518518 | | | |

Appendix 8 Analysis of variance of data for tomato yield (fruits weight) exposed to repeated application of neem formulations and mitac on two-spotted spider mites population in the greenhouse.

| Source | DF | SS | MS | F Value | Pr |
|---|---|---|---|---|---|
| Treatment | 8 | 554.314193 | 69.289274 | 16.77 | 0.0001 |
| Error | 655 | 2705.544598 | 4.130602 | | |
| Corrected total | 663 | 3259.858791 | | | |