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EFFICACY OF SOME PLANT OILS AND GROWTH REGULATORS ON TWO-SPOTTED SPIDER MITE

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ABSTRACT

Two-spotted spider mite, *Tetranychus urticae* Koch, is one of the most damaging pests of many economically important crops. The acaricidal, ovicidal and nymphal activity of three essential plant oils viz. neem, mahagoni, castor and two insect growth regulators viz. buprofezin and lufenuron + Emamectin benzoate were evaluated against *T. urticae* under laboratory conditions and in the potted plants. Results showed that mahagoni oil (100%) and lufenuron + emamectin benzoate (100%) performed the most efficient acaricidal agent against *T. urticae* in the laboratory conditions. Mahagoni oil also showed the highest ovicidal efficacy (77.2%) and lufenuron + emamectin benzoate (74.6%) showed the second highest ovicidal efficacy against *T. urticae*. Castor oil showed the lowest percent of ovicidal efficacy (48.1%). In the case of the nymph, the highest average mortality was observed in mahagoni oil (55.4%) followed by lufenuron + emamectin benzoate (52.8%). The lowest nymphal mortality was observed in castor oil (31.3%). In potted bean plants, all the treatments showed a significant reduction in the adult population over control up to 21 days. The adult *T. urticae* population's survival rate progressively declined throughout the length of the days after treatment (DAT) for each treatment up to seven days, then slightly climbed up to 21 days. However mahagoni oil (77.8%) and lufenuron + emamectin benzoate (72.2%) showed the highest mite mortality compared to all other treatments. Therefore mahagoni oil and lufenuron + emamectin benzoate were found to be the most effective to control *T. urticae.*

Keywords: Country bean, *Tetranychus urticae*, mortality, plant oils.

Introduction

The two-spotted spider mite was originally described from European specimens. Since 1900 the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), has become an increasingly important agricultural pest and is a worldwide pest of many plant species including several economically important agricultural crops. It is one of the most damaging polyphagous pest and attacks more than 1000 plant species of vegetables, fruits, crops, and a wide variety of ornamentals are among its host plants belonging to about 140 plant families (Grbic *et al*. 2011). Some vegetables are grown year-round, while others are produced during specific growing seasons. They are important food considering the aspects of nutritional, financial as well as food security in Bangladesh. According to Rincon *et al.* (2019), the price of losses caused by this pest in crops like beans, citrus, cotton, avocado, apples, pears, plums, and many more horticultural and decorative crops is expected to be above 4500 USD per hectare.

The two-spotted spider mite prefers the hot, dry weather of the summer and fall months, but may occur anytime during the year (Fasulo and Denmark 2009). Overwintering females hibernate in ground litter or under the bark of trees or shrubs. Season and host plants have an impact on the longevity and fecundity of this mite (El-Taj *et al.* 2016). Adults and nymphs consume the sap from the underside of leaves, which causes yellowing and staining (Reddy and Kumar 2006). Feeding close to the midrib and plant veins typically results in a 50–100% yield loss (Kumar *et al*. 2010). In cases of severe infestation, the mite creates webbing on the leaves which causes the damaged leaves to dry out and fall off (Reddy *et al*. 2014). In greenhouse conditions, it causes economic damage to high value crops in severe infestation. The damaged cells appear as yellowish white spots and chlorophyll is destroyed from the upper surface of the leaf. Continued feeding causes a stippled-bleached effect and later, the leaves turn yellow, grey or bronze. Mite not only causes direct damage to plants by leaf defoliation and burning but also causes indirect damage to plants due to decrease in photosynthesis and transpiration (Brandenburg and Kennedy 1987).

The control of spider mites is a challenge and difficult due to their short life cycle. Conventionally, spider mites have been controlled by different systemic chemical pesticides but quick development of pesticide resistance by the mite was reported (Van Leeuwen *et al*. 2010). Moreover, the use of synthetic chemicals has resulted in serious environmental problems and has been a threat to human life (Kim *et al*. 2005). Therefore, alternative strategies such as the use of different bio-control agents, essential plant oils and insect growth regulators need to explore for their acaricidal activity against the *T. urticae* in order to reduce the chemical acaricides that are currently being used.

The essential oils (EOs) had pesticidal activities due to the presence of monoterpenes, diterpenes and sesqui terpenes (Mohamed and Alotaibi 2023). Many plant derived essential oils can be a potential alternative for mite control, because some of them are selective, biodegradable, and have few effects on non-target organisms and the environment (Giunti et al. 2022). They usually exert multiple types of beneficial properties such as repellence, antifeedant activity, growth regulatory activity and toxicity to many insect and mite pests Alexenizer and Dorn 2007). Insect growth regulators (IGRs) are substances that adversely affect insect growth and development (Tunaz and Uygun 2004). Buprofezin and lufenuron, which are potential chitin synthesis inhibitors, were found effective against many arthropod pests including mites (Kavya *et al*. 2015). However, reports on the efficacy of plant oils and IGRs against the populations of *T. urticae* were rarely found. Considering that the plant oils and IGRs have been not focused on spider mites, or in their acaricidal properties, the present research work was under taken to search a potential plant oil and insect growth regulator with their acaricidal, ovicidal and nymphal efficacy against two-spotted spider mite.

Materials and Methods

The study on the efficacy of some plant oils and insect growth regulators against *Tetranychus urticae* was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period of July to December 2022.

Mite collection and rearing: The adult *Tetranychus urticae* was collected from an infested bean field of HSTU campus, Dinajpur, Bangladesh from August to September 2022. The colony was maintained without application of any acaricides or pesticides on country bean plants reared on the bean leaves in the laboratory.

Table 1. List of treatments used in bioassay

Treatments	Trade name	Active ingredients	Nature of chemical	Manufacturar/ sources	Recommended dose / L water
T_1	Neem oil	Azadirachtin	Antifeedant and repellent	Market	2.0 ml
T_2	Mahogany oil	saponin	Antifeedant and repellent	Market	2.0 ml
T_3	Castor oil	Ricicin	Antifeedant and repellent	Market	2.0 ml
T_4	Award 40 SC	Buprofezin	Contact and stomach	Square pharmaceuticals company Limited	0.5 %
T_5	Lumectin 10 WDG	Lufenuron (5%) + Emamectin Benzoate (5%)	Contact and systemic	Haychem (Bangladesh) Limited	0.5 ml
T_6	Control	-	-	-	-

Collection of plant oils and insect growth regulators: Plant oils namely neem, *Azadirachta indica* A. Juss, Mahogany, *Swietenia mahagoni* L, Castor, *Ricinus communis* L. and insect growth regulators were collected from local market at Dinajpur town, Bangladesh. Their detailed information is given in the Table 1. All the treatments were mixed with tap water at the concentrations recommended by the manufacturers. The acaricidal effect of these treatments against *T. urticae* was studied both in the laboratory and in the potted bean plants.

Topical application of treatments to adult females of *T. urticae*: A group of eighty *T. urticae* females (48 hours old) were randomly selected from culture and transferred equally to four fresh bean leaf discs (3 cm in diameter) placed adaxial side on moistened cotton in a Petri dish. Those mite-leaf discs were sprayed with the selected acaricidal suspension (2 mg cm^{-2}) using hand sprayer. The Petri dishes were left naked to avoid mite mortality due to any gassing effect of the tested treatments. A control test was maintained using tap water. Mites were considered dead as their appendages not move when prodded with a fine brush at 6, 12, 18, 24, 30, 36, 42 and 48 hours after treatment (HAT). An stereomicroscope was used to record the number of alive and dead mites. Four leaf discs serving as replicates were maintained for each treatment. Mortality data were corrected using the Abbott's correction formula (Abbott 1925).

Topical application of treatments to eggs of *T. urticae*: Bean leaf discs (3 cm diameter) were used as a substrate to ovipositor. Four leaf discs were used for each treatment and 20 female mites were placed on upside down leaf discs which were attached with wet cotton in a plastic Petri dish (9 cm diameter) and allowed to stand for 6 hours for laying eggs. After then, the adults were removed and the eggs were checked under a stereomicroscope to ensure that at least 20 eggs less than 24 hours old had been laid on each leaf disc. The leaf discs with eggs were treated with selected oils and growth regulators concentrations of 2% and 0.5ml/L with the help of a hand sprayer and allowed to dry. Control units were sprayed with tap water as a control. The number of hatched and non-hatched eggs was recorded after seven days. A stereomicroscope was used to record the number of hatched and non-hatched eggs. All females and eggs were tested under controlled conditions (25±2°C, 65±10% RH, and 16: 8 L: D).

Topical application of treatments to nymph of *T. urticae*: Bean leaf discs were placed on moistened cotton in a Petri dish. Ten moving nymphs were placed on the ventral surface of each leaf discs. The nymph with leaf discs was sprayed with the selected oils and growth regulators. A control group was maintained by spraying tap water only. Death individuals were recorded after 24, 48 and 72 hours of spraying.

Toxicity assessment in potted bean plants: The experimental area (300 L × 180 W × 300 H cm) was covered under a natural photoperiod with a thrips-proof (196 mesh) screening. The bean seeds variety of BARI seem 1 were sown in plastic containers (20 D ×20 H cm), filled with sandy loam soil and farm yard manure (1:1). Plants were allowed to grow with bamboo supported sticks. The bean plants have been fertilized and the water is supplied properly. No plant protection measures were applied throughout the study period. One month before the beginning of the study, a four-week old potted bean plants was infested with twenty adult gravid females of *T. urticae* obtained from the stock culture. All the treatments were applied at the field recommended concentration using knapsack sprayers (model ML10) under a fume hood until run off. Only water was sprayed in the control treatment. The spray volume per plant was 200 ml. Prior to treatment (day 0), randomly selected three infested leaves in each plant was observed as the typical signs of infestation (chlorotic spots). These leaves were marked, and the number of all *T. urticae* adult females on each was counted with a magnifying glass. On days 1, 3, 7, 14 and 21 after spraying, counting was repeated on those leaves. A complete randomized design (CRD) was used with six treatments and three replications.

Statistical analyses: The mortality percentage was calculated by using Abbott's corrected formula (Abbott 1925). Data were analyzed with ANOVA followed by LSD test at $p<0.05$ using statistix 10 software. Graphical works were done with excel program.

Results and Discussion

Acaricidal activity of neem, mahogany, castor oil, buprofezin and lufenuron + emamectin benzoate were tested against *T. urticae* at different time interval and are shown in Figure 1. Results showed that the mahogany

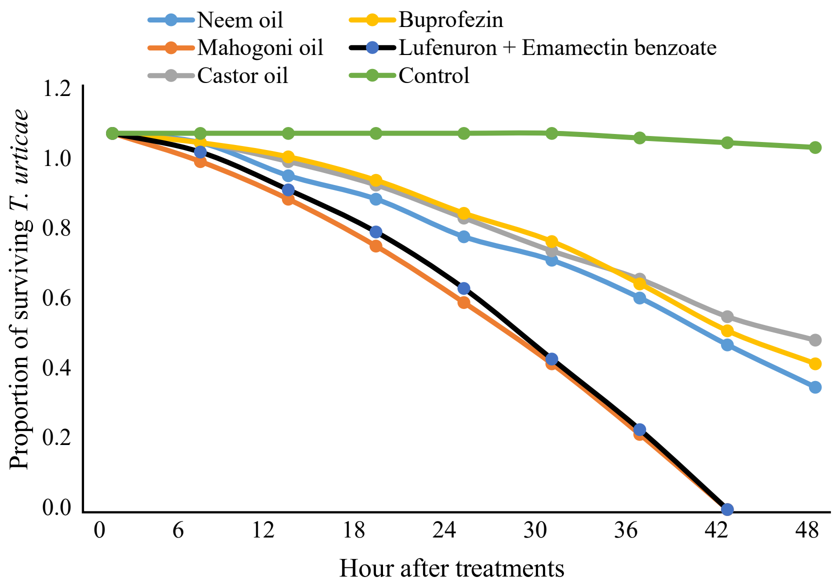

Figure 1. Survivorship of *T. urticae* adult females exposed to different treatments in topical application

oil and lufenuron + emamectin benzoate were the most potent miticide which caused the highest mortality to adults of *T. urticae* with 100% mortality within 42 hours of exposure. The second most toxic was found from neem oil with 67.5 % mortality in adult *T. urticae* (Figure 1). The mortality percentage was increased proportionally within hours after treatments. There was no mortality observed in control treatments up to 30 hours of exposure but low mortality (1.25%) was found after 48 hours of exposure. On the basis of adult mortality percentage, the order of the toxic effect of three botanical oils and two growth regulators against *T. urticae* adult were found as mahogany oil > lufenuron + emamectin benzoate > neem oil > buprofezin > castor oil.

From the above results, it is found that all the treatments showed a different level of adult mortality but mahogany oil and lufenuron + emamectin benzoate showed the best result with the highest performance. This finding is in agreement with the result of Uddin *et al.* (2014). They found that mahogany oil has the highest acaricidal activity followed by neem and eucalyptus oil. In another study, El-Zalabani *et al.* (2012) stated that ethanoic extracts of leaves and stem bark of *S. mahagoni* and *S. macrophylla* has strong acaricidal activity against *Varroa destructor* with 90% mortality.

The results presented in Table 2 that all the treatments showed significantly ($P<0.05$, $df=5$, $F=57.60$) different percent egg hatching over control. The lowest percent egg hatching (22.5%) was observed when the eggs were treated with mahogany oil which also showed the highest ovicidal efficacy (77.2%). The second lowest percent egg hatching (25.0%) was observed when the eggs were treated with lufenuron + emamectin benzoate and neem oil (33.7%) which was statistically similar. On the other hand, significantly the highest percent of egg hatching (51.2%) was found from the treatment of castor oil with showed the lowest percent of ovicidal efficacy (48.1%). In the control treatment significantly the highest percentage of egg hatching was observed (98.7%).

Above results from Table 2 indicated that mahogany oil showed the highest ovicidal efficacy which is significantly different from other treatments. Mahogany oil and lufenuron + emamectin benzoate significantly differ from control and other treatments. Based on the results, the effects of plant indicated the toxicity of the plant oils and growth regulators against the egg of *T. urticae* and the highest mortality percent (77.2%) was found in mahogany oil and castor oil showed the lowest mortality (48.1%).

The obtained results are in agreement with the results obtained by Abdelgaleil and Nakatani (2003). They stated that the limonoids in the mahogany products have also been found effective against cotton leaf worms. EI-Rahman *et al.* (2016) stated that oils of *A. indica* comes in

Table 2. Egg hatching of *Tetranychus urticae* exposed to different plant oils and insect growth regulators by direct spray and water- treated control with the corresponding efficacy.

Treatments	% Egg hatching (Mean±SE)	% Efficacy over control
Neem oil	33.7±5.5 cd	65.8
Mahogany oil	22.5 ±3.2 e	77.2
Castor oil	51.2±4.2 b	48.1
Buprofezin	38.7±2.4 c	60.7
Lufenuron (5%) + Emamectin Benzoate (5%)	25.0 ±4.1 de	74.7
Control	98.7±1.2 a	-
CV (%)	16.5	
Level of significance	0.01	
CD	11.07	

Means within a column followed by different letters are significantly different at 5% level of probability by LSD.

the category of the least effective compound on the eggs of *T. urticae* which conformed to the present findings. Abd EI-Wahab (2003) indicated that castor oil treatment appeared to be the most effective against the population of *T. urticae* than soybean oil on cucumber.

Effects of different oils and growth regulators at different time intervals on the mortality of the *T. urticae* nymph are presented in Table 3. Percent mortality at 24 HAT was statistically different among the treatments ($p <0.05\%, df =5, F =17.4$). The highest nymphal mortality (25%) was observed when applied mahogany oil and the lowest mortality (10%) was found in castor oil. No mortality was observed at control treatment at 24 HAT. After 48 HAT, significantly the highest mortality was observed from the treatments of mahogany oil (55%) and lufenuron + emamectin Benzoate (55%) followed by neem oil (45%) which were found statistically similar. But the lowest mortality was observed from the treatment of castor oil (32.5%) followed by buprofezin (35%) which is statistically identical but statistically different from other treatments. There was no mortality observed in control treatment also at 48 HAT. At 72 HAT the highest mortality was found when applied mahogany oil (86.4%) and the lowest one was castor oil (51.4%). Average mortality indicated that significantly the highest mortality was observed in mahogany oil (55.4%) followed by lufenuron + emamectin Benzoate (52.8%) which was statistically similar between those two treatments. The lowest average mortality was observed in castor oil (31.3%). The mortality

Table 3. Percent mortality of *T. urticae* nymph exposed to different treatments in tropical application

Treatments	Percent mortality (Mean±SE)			
	24hr	48hr	72hr	Average
Neem oil	17.5 ±2.5 b	45.0 ±2.9 b	70.3±2.6 bc	44.2 abc
Mahogany oil	25.0 ±2.9 a	55.0 ±2.9 a	86.4±2.8 a	55.4 a
Castor oil	10.0 ±0.0 c	32.5 ±2.5 c	51.4±2.6 d	31.3 c
Buprofezin	15.0 ±2.8 bc	35.0 ±2.9 c	62.2±2.7 c	37.4 bc
Lufenuron (5%) + Emamectin Benzoate (5%)	25.0 ±2.9 a	55.0 ±2.9 a	78.3±4.5 ab	52.8 ab
Control	0.0 d	0.0 d	7.5 ±2.5 e	2.5 d
CD (p = 0.05)	6.7	7.6	9.1	
CV (%)	29.6	13.8	10.3	

Means within a column followed by different letters are significantly different at 5% level of probability by LSD. ±SE: indicates standard error, CD: critical difference test.

of neem oil and buprofezin was found 44.2% and 37.4% respectively. A little average of nymphal mortality was found in the control (2.5%) treatment.

From the above results, it is clear that different treatments showed remarkable nymphal mortality at a different level but mahogany oil showed the best performance. The present findings are in agreed with those of Uddin *et al.* (2014) who found that mahogany oil showed effective acaricidal activity against *T. urticae.* Satti and Elamimm (2012), conducted an experiment to evaluate the insecticidal activities of several extracts prepared from two meliaceous plants, viz., mahogany and neem, against the 3rd instar larvae of *T. granarium.* Mahogany leaves exerted better actions than those of neem leaves. In present study, mahogany oil showed better performance than neem oil. Mohammed *et al*. (2018) proved that castor oil has a moderate mortality proportion against *T. urticae* with high concentrations.

All treatments showed a significant reduction in the female population over control up to 21 days (Table 4). Survival of the population of female *T. urticae* decreased gradually with the progress of days after treatment (DAT) of each treatment as compared to control. Lufenuron + emamectin benzoate, mahogany oil, castor oil, buprofezin and neem oil showed less mortality effect on day 1 after treatment where the mortality percentage was 28.5%, 25.7%, 23.5%, 19.7% and 18.2%, respectively. One week later, lufenuron + emamectin benzoate (67.0%) and mahogany oil (62.5%) showed >50% mortality. At 14 DAT, all treatments showed significantly different mortality rates as compared with the untreated control. No significant difference was observed among neem oil (55.1%) and buprofezin (54.1%) (Table 4). At 21 DAT, the mortality caused by mahogany oil, lufenuron + emamectin benzoate and neem oil was 77.8%, 72.2%and 60.7%, respectively which was significantly different from the untreated control and considerably higher than the other treatments. However, mortality rate of buprofezin and castor oil was not significantly different from that of mahogany oil, lufenuron (5%) + emamectin benzoate (5%), and neem oil (Table 4).

The effectiveness of three essential oils and two insect growth regulators varies among the treatments on the species of mites. However, we observed that from three oils the mahogany oil had the greatest direct effect on *T. urticae*, followed by neem and castor oils. From the two growth regulators, lufenuron + emamectin benzoate showed better performance than buprofezin. Our results are agreed with those of Bamaiyi *et al.* (2006) studied that mahogany seed oil for its effectiveness to control *Callosobruchus maculatus*on stored cowpea. Over the past 30 years, a large number of laboratory and field research have been carried out utilizing various plant extracts or products that have the potential to be used as botanical pesticides to control agricultural mites (Jia *et al.* 2011, Pavela 2016). Among the plant oils tested, mahogany oil was performed significantly better than neem and castor oils. The possible explanation for the superior performance of mahogany oil could be the inclusion of some unique natural elements, or phytochemical studies

Table 4. Mean density of *T. urticae* adult female before spraying (0 day) and after 1, 3, 7, 14 and 21 days after words and the corresponding efficacy

Treatments	Number of adult mites (±SE) survived per leaf					
	Day 0	Day 1	Day 3	Day 7	Day 14	Day 21
Neem oil	24.5±1.7 a	20.7±1.6 ab (18.2)	20.7±1.6 ab (25.1)	8.9±1.1 bc (36.7)	5.1±0.6 c (55.1)	4.1±0.5 c (60.7)
Mahogany oil	23.6±0.8 a	18.4±0.8 b (25.7)	12.3±1.1 bc (39.7)	5.3±0.8 d (62.5)	2.9±0.5 d (74.9)	2.2±0.2 d (77.8)
Castor oil	20.5±0.9 a	19.1±0.6 ab (23.5)	15.0±0.6 abc (28.6)	9.8±0.6 b (30.3)	7.7±0.6 b (32.7)	6.5±0.3 b (37.5
Buprofezin	23.3±1.1 a	20.1±0.7 ab (19.7)	14.4±0.6 abc (30.8)	7.8±0.1 c (44.4)	5.3±0.6 c (54.1)	4.5±0.4 c (56.1)
Lufenuron (5%) + Emamectin Benzoate (5%)	22.7±1.2 a	18.0±1.2 b (28.5)	12.1±1.2 c (42.0)	4.6±0.7 d (67.0)	3.0±0.5 d (74.4)	3.1±0.6 cd (72.2)
Control	21.9±2.2 a	22.8±2.1 a	16.1±1.1 a	14.1±0.3 a	11.5±0.2 a	10.6±0.9 a

Means within a column followed by different letters are significantly different at 5% level of probability by LSD. ±SE: indicates standard error. Values within parentheses are percentage over control.

that have made it possible to identify and isolate various secondary metabolites that are toxic to *T. urticae*, such as tannins, saponins, and phenolic acids (Sukardiman and Ervina 2020). El Zalabani et al. (2012) indicated that both leave and bark extracts of *S. mahogany* at 500 ppm were the most effective against varroa mite with 90% of mortality. The two insect growth regulators (IGRs), buprofezin and lufenuron were found moderately effective against *T. urticae*. However, IGRs are considered to be safe for beneficial organisms (Ishaaya *et al*. 2001), and the selected IGRs could be considered for the development of an IPM program for *T. urticae*.

Conclusion

Mahogany oil and lufenuron + emamectin benzoate were the most potent miticide which caused the highest mortality to adults of *T. urticae* with 100% mortality within 42 hours of exposure. The mortality percentage of *T. urticae* egg indicated that mahogany oil showed the highest mortality (77.2%) among all the treatments. In the potted been plants the highest mortality was found in mahogany oil (77.8%) and the lowest showed in castor oil (37.4%) at 21 days. Therefore, mahogany oil and lufenuron + emamectin benzoate were the most effective natural alternatives to synthetic chemical pesticides for the control of *T. urticae* in vegetable field.

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