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EVALUATION OF SOME BOTANICAL EXTRACTS AGAINST TWO-SPOTTED SPIDER MITE (TETRANYCHIDAE: *TETRANYCHUS URTICAE* KOCH) UNDER LABORATORY CONDITION

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ABSTRACT: Two-spotted spider mite (TSSM) (*Tetranchus urticae* KOCH), is the most devastating polyphagous sap-sucking pests of many vegetables, fruits, cereal crops, and ornamental plants. TSSM is the major pest of potato in Eastern Hararghe, Ethiopia. The present study was conducted to evaluate the potential of *Azadirachta indica* seed and leaf oil, *Tagetes minuta* leaf oil and their leaf aqueous extracts against TSSM in the plant protection laboratory of Haramaya University. Amitraz 20 EC was used as a standard check in the experiment. The experiment was arranged in a completely randomized design (CRD) in four replications. Treatments were applied on adults of TSSM both by spray and dipping methods, while only dipping method was used on the eggs of TSSM. The number of TSSM dead adults was recorded for three days (72 hrs) at 24 hrs intervals, while egg mortality was recorded 12 days after treatment application. The mortality percentage was corrected using Abbot's formula before data analyses. Significant differences (P<0.05) were observed among the treatments both in adult and egg mortality of TSSM. The highest mortality was obtained with Amitraz 20 EC (95.5-100%), followed by *A. indica* seed (90.125-94.95%) and *T. minuta* leaf oil (78.5-84.5%) in spray and dip bioassay 72 hrs after treatment application. The least effective treatments were *A. indica* leaf aqueous leaf extracts (AqE) (56.125%) and *A. indica* leaf oil (55.45%) in spay and dip bioassay 72 hrs after treatments application. The highest mortality of TSSM eggs was obtained from the application of Amitraz 20 EC and *A. indica* (92.28%), followed by *T. minuta* leaf oil (83.74%). The lowest mortality (21.34%) and highest hatching (78.66%) of TSSM eggs were recorded from the application of leaf aqueous extracts. The study also revealed that mortality of TSSM was clearly time-dependent i.e. mortality increased proportionally with increase in time after treatment application. The study revealed the promising efficacy of *A. indica* seed oil and *T. minuta* leaf oil that were comparable to Amitraz 20 EC in the management of TSSM under laboratory condition.

Key words/phrases: Botanicals, Management, Polyphagous, Sap-sucking pest, Synthetic Pesticide, *Tetranychus urticae*

INTRODUCTION

Two-spotted spider mite (TSSM) (*Tetranchus urticae* KOCH: Tetranychidae) is one of the most devastating polyphagous sap-sucking pest of many vegetables, fruits, cereal crops, and ornamental plants. It feeds on and caused significant damage to more than 1,100 plant species, of which over 100 are agriculturally important crops (Bensoussan *et al.*, 2016). The infestation of TSSM results in the overall decline of plant growth and its yield (Rakha *et al.*, 2016; Raghavendra *et al.,* 2017).

TSSM was first noticed in Eastern part of Ethiopia on *Solanum tuberosum, Datura stramonium, Solanum indicum, S. nigrum, Solanum lycopersicum, Melia azedarach* and *Catha edulis* (Muluken Goftishu *et al.,* 2016). It became a major pest in 2014-2015 cropping season following shortage of rainfall or drought that happened in the region due to Elino (Gebissa Yigezu *et al.*, 2019). It caused potato yield losses of up to 100% in this region (Mohammed Ibiro *et al.*, Personal communication, 17 February, 2019). Eastern Ethiopian farmers applied different synthetic pesticides to reduce damage from this devastating pest. However, reliance on synthetic chemicals to control the pest did not do more than giving temporary relief (Grbic *et al.*, 2011). Synthetic pesticides have also had environmental and human health related drawbacks. The reliance on the ineffective pesticides also caused the TSSM resurgence.

Applications of botanical pesticides are routine practices of farmers against arthropod pests. Botanicals are naturally occurring chemicals extracted from plants and used as pesticides (Sithisut *et al.*, 2011). It is safer and biodegradable than synthetic pesticides. It could minimize the risk of ozone depletion, neurotoxic, carcinogenic, teratogenic and mutagenic effects on non-target organism. It also avoids development of multi-resistance in insects which is the case with the application of synthetic pesticides over time (Regnault-Roger *et al.*, 2012).

Many components of botanical pesticides affected varieties of insect pests as may be repellent, deterrents/anti-feedants, ovicides, chemosterilants, attractants, toxicants, oviposition inhibitors and growth retardants (Regnault-Roger *et al.*, 2012). Botanical pesticides might be one of the main strategies in plant protection in the fight against insect pests (Erdogan *et al.*, 2012). Some work has also been conducted on selecting effective and relatively safer miticides to be used alone or in integration with some botanicals. Therefore, it is important to screen locally available botanical plants against TSSM that could add

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to the effective insecticides menu. Identification of such botanical pesticide will enable to use in alteration or integration with synthetic pesticides. Such alteration or integration delays pesticide resistance in the pest and consequently increase the shelf life of effective pesticides.

In Eastern Haraghe, farmers suffered from TSSM attack on potato, tomato, Khat, cucumber, beans and many other crops. The damage and loss of potato by TSSM had reached up to 100% during dry season in 2016/17 (Muluken Goftishu *et al.*, 2016). Therefore, this research was initiated to evaluate the potential of some selected botanicals against TSSM under laboratory condition.

MATERIALS AND METHODS

Description of the study area

The study was conducted at Haramaya University Plant Protection laboratory in October 2018/2019. Haramaya University is 550 km away from Addis Ababa to the East, and 40 and 20 km away from Dire Dawa and Harar cities, respectively. The experiment was conducted in the laboratory at the room temperature of 24 ± 2 ^{0}C and 60-65% RH; photoperiod was 12:12 hrs day and night (L:D).

Collection and Preparation of Botanicals

A. indica seed and leaves were collected from Dire Dawa Tonny farm, while *T. minuta* leaf was collected from Haramaya district. Both plants were selected among the many botanicals for their better performance as pesticide and availability. After collection, the botanicals were taken to the Plant Protection Laboratory of Haramaya University and dried under shade. The seed coat of *A. indica* seed was broken manually by rotating a glass beaker on a bench to separate the seed. The prepared seed samples were then ground by using High-Speed Smashing Machine and sieved by 0.25 mm pore size mesh.

Extraction of Plant Materials and Isolation of Oils

A. indica seed, *A. indica* and *T. minuta* leaf were ground and oils were extracted from 30-grams of the powders. The powders were filled into empty clean thimbles, covered by defeated cotton, placed and fixed into the Soxhlet extraction apparatus following the work of Stoll (2000). Petroleum ether of a 250ml volume was added into a prepared clean sterilized conical glass flask. It was used for mixing and dissolving the powder. Finally, the apparatus was connected to electricity that was put on for eight hours to extract oils. After 8 hours of extraction, the oils were poured into the prepared sterilized conical glass flasks and fitted to the Rotary Evaporator apparatus at 50°C to evaporate the remained petroleum ether in order to get pure oils. Finally, the oil was stored in a refrigerator until the experiment was started following the work of Dawit Kidane and Bekele Jembere (2010) and Kifle Gebre-Egziabiher *et al.* (2016). The oils were applied at the rate of 3% after dissolving in 100ml of tap water.

Preparation of Aqueous Extracts

T. minuta and *A. indica* leaf powder were already prepared and stored as stated above. The aqueous extracts of *T. minuta* and *A. indica* leaf powder was then added to distilled water. The powder was measured using sensitive balance and 70gm of it was taken and mixed in one liter of distilled water. The mixture was added into 5lt sterile conical glass flasks and sealed tightly by aluminum foil and fitted to Shaking Incubator with the 80 shakes per minute for 24hrs to make final solution. After 24hrs of shaking, the incubator was siphoned off. The solution was filtered through Whitman filter paper for 1hour into the 2.5lt sterile conical glass flasks. This filtered solution was stored and used as a stock solution. The stock solution was applied at the rate of 5% (Mmbone *et al.*, 2014) after further dissolving in 100ml of tap water.

Two-spotted Spider Mite Population

A two-spotted spider mite population was collected from infested wild plants of *S. indicum* and potato tubers stored at Haramaya University, Potato Research Program. The two spotted spider mites were then reared and maintained at Haramaya University Greenhouse on Common Bean plants (*Phaseolus vulgaris* L.) from September 2018 to March 2019. Fresh bean plants were provided to the TSSM at weekly intervals.

Preparation of Leaf Disks

Laboratory Bioassay Procedure- Fresh, untreated, and un-infested leaves were collected from *P. vulgaris* plants and taken to the Plant Protection Laboratory, Haramaya University. The leaves were washed thoroughly by distilled water and air-dried. After then a 4 cm x 4 cm bean leaf disc was prepared. Cotton wool was washed by detergent (soap) and completely air-dried. In this preparation a Cotton Wool was placed on the plate of Petri dishes gorge (under the leaf disc) for moistening, maintaining turgidity and avoiding drying of the bean leaves. *P. vulgaris* leaves were provided as a food for TSSM and Tissue paper was used for controlling the escape of TSSM from the experimental units/Petri dishes. *A. indica* and *T. minuta* extracts were sprayed on TSSMs on the top of perforated glass Petri dishes for the leaf spray and dipping trials (Table 1). Amitraz 20EC was used as a standard check.

The experiment was conducted to evaluate the miticidial activity of *A. indica* seed and leaf oils, *T. minuta* leaf oils and their aqueous extracts against TSSM adults and eggs. The experiment was arranged in a completely randomized design (CRD) in four replications. The methods of application were leaf spray and dipping. Three days old thirty adult TSSM were sprayed on separate Petri dishes for the spray bioassay, while the same age and number of adults were dipped for the dipping bioassay. The egg bioassay experiment began with two gravid females that were placed on leaf disks on each Petri dish to lay eggs for two days. After two days, ten eggs were counted and left in each Petri dish and the rest discarded. The Petri dishes containing ten eggs were sprayed with the specified rate of pesticides (Table 1).

The treatments were applied (0.5ml by volume) using a spray bottle from a distance of 20 cm and at an angle of 90 degrees following the work of Tesfay *et al*. (2016). Similarly, thirty adults TSSM were dipped in 100ml of the prepared dissolved solution of pesticides for five seconds by holding with forceps. After dipping, it was placed on the prepared leaf disks described above, while the control was dipped in 100ml of tap water. The remaining TSSM adults (alive) soon after dipping were counted under a dissecting stereo microscope and recorded.

The results were assayed following the work of Erdogan *et al*. (2012). Each time, if the TSSM adults became incapable of moving appendages, or unable to maintain normal attitudes like leg coordination, or any signs of life when probed with a fine brush then it was considered dead (Panella *et al*., 2005). The eggs were also considered dead when shrunk, broken, changed the normal color and dried.

Table 1. List of botanicals/pesticide and their rates

Treatments	Rates dissolved in 100mL of tap water	Rate applied per Petri dishes in 0.5 ml
A. indica aqueous leaf extracts (AqE)	5%	25 µL
A. indica leaf oil	3%	15 µL
A. indica seed oil	3%	15 µL
T. minuta AqE	5%	25 µL
T. minuta leaf oil	3%	15 µL
Amitraz 20EC	60 µL	0.3 µL
Untreated control	100ml of tap water	0.5ml

Data Collection

The number of dead TSSM was counted and mortality was recorded every 24 hours for three consecutive days (72 hours) after spraying, while the mortality of TSSM eggs was recorded 12 days after treatment application.

Data Analysis

Percent mortality of TSSM was calculated using Abbott (1925) formula as follows:

$$\text{Mortality \%} = \frac{\text{The died number of TSSM adults and eggs counted after treatment application}}{\text{Total number of TSSM adults and eggs counted before treatment application}} * 100$$

Then the corrected mortality data were statistically analyzed using SAS version 9.0 (SAS, 2002). The data were subjected to ANOVA in general linear model (GLM) procedure. A 5% level of significance was used for all analyses and significant means were separated using the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Bioassay of some Botanical Pesticides against TSSM: Leaf Disks Spray

Mean percent adult mortality of TSSM showed significant difference compared to the untreated check (P<0.05%) (Table 2). Among the botanical treatments, *A. indica* seed oil caused the highest percent adult mortality of TSSM followed by *T. minuta* leaf oil. However, there was no significant difference among *A. indica* seed oil, *T. minuta* leaf oil and Amitraz 20EC 24 hrs after treatment application. But, they significantly differed from *A. indica* leaf oil and A. *indica* leaf AqE, and *T. minuta* leaf AqE 24hrs after treatment application. The least mortality (33%) of TSSM adults was recorded from *A. indica* leaf AqE among the botanical treatments followed by the untreated check which was 11% mortality.

The highest cumulative mean percent adult mortality (84%) of TSSM was recorded from the application of Amitraz 20 EC followed by *A. indica* seed oil (79.41%) and *T. minuta* leaf oil (73.75%) with no significant difference amongst them *48hrs after spray*. However, they differ significantly (P<0.05) from the application of *T. minuta* leaf AqE, *A. indica* leaf oil, *A. indica* leaf AqE and the untreated check (P<0.05). The *A. indica* leaf AqE 70g/L caused the least percent mortality of TSSM adults (46.75%) amongst all the botanical treatments followed by the untreated check (17.5%) (Table 2).

Amitraz 20 EC caused the highest percent mortality (95.5%) of TSSM adult followed by *A. indica* seed oil (90.13%), while *A. indica* leaf AqE caused the least mortality (56.175%) 72 hrs after spray. The mortality of adults increased with the length of time (hours).

It can be concluded from the leaf disc spraying laboratory experiment that *A. indica* seed oil and *T. minuta* leaf oil 3% had comparable miticidal effect with Amitraz 20 EC (standard check) throughout the observation period.

Table 2. Mean percent mortality of two spotted spider mite (TSSM) adults after 24, 48 and 72 hrs of leaf disc spray.

Treatments	Percent mean mortality hours after treatment application		
	24 hrs	48 hrs	72 hrs
T. minuta leaf oil 3%	66.275[a]	73.750[a]	78.500[b]
T. minuta leaf AqE 70g/L	37.000[b]	56.250[b]	60.500[c]
A. indica seed oil 3%	71.125[a]	79.425[a]	90.125[a]
A. indica leaf oil 3%	42.125[b]	56.725[b]	71.450[bc]
A. indica leaf AqE 70g/L	33.000[b]	46.750[b]	56.175[d]
Amitraz 20 EC 20µL	64.000[a]	84.000[a]	95.500[a]
Control	11.000[c]	17.500[c]	22.000[e]
CV	11.23	7.55	7.18
LSD	11.97	10.28	11.17

Means followed by the same letter (s) with in a column are not significantly different from each other at 5%, LSD.

Leaf Dip Bioassay

The Cumulative mean mortality percentages of TSSM adults dipped in pesticides under laboratory showed significant difference (p<0.05). However, Amitraz 20 EC, *A. indica* seed oil, and *T. minuta* leaf oil did not differ significantly in killing TSSM 24, 48, and 72 hours after leaf dipping (P<0.05). Numerically, the highest percentage mortality of TSSM adults were recorded from Amitraz 20 EC (72%), followed by *A. indica* seed oil (71.13%) and *T. minuta* leaf oil (63.78%). Moreover, there was no significant difference among *A. indica* leaf oil, *A. indica* and *T. minuta* aqueous leaf extracts (AqE) (p<0.05). Among the treatments, the least percent adult mortality of TSSM was recorded from the application of *A. indica* leaf oil (33.53) followed by the untreated check (Table 3).

Similar trend was also followed 48hrs after dipping. The cumulative mean percent adult mortality of TSSM was significantly different (P<0.05). Amitraz 20 EC caused the highest percent adult mortality of TSSM (99%) followed by *A. indica* seed oil (89.43%) and *T. minuta* leaf oil (68.75%), while *A. indica* leaf oil (47%) caused the least percent mortality followed by the untreated check (19.5%).

Moreover, the effect of the treatments were more or less constant throughout the times observations as they also significantly differed 72 hours after leaf disc dipping (P<0.05). Amitraz 20 EC caused total mortality of TSSM adults (100%), followed by *A. indica* seed oil (94.95%), while *A. indica* leaf oil (55.45%) caused the least mortality, followed by untreated control (19.5%) (Table 3).

It can be concluded from the leaf disc dipping experiment that *A. indica* seed oil, *T. minuta* Leaf AqE 70g/L *A. and A. indica* Leaf AqE 70g were the most promising botanical miticides under laboratory conditions almost throughout the observation period. In other words, both the leaf disc spraying and dipping methods gave almost similar efficacy to Amitraz 20EC or the standard check used in the experimentation. If similar effect can obtained under field conditions, these miticides can replace Amitraz 20EC or be used in alternate applications to delay pest resistance and also reduce cost of production.

Table 3. Mean percentage mortality of two spotted spider mites (TSSMs) adults' after 24, 48 and 72hrs of leaf disc dipping.

Treatments	Percent mean mortality hours after leaf		
	24 hrs	48 hrs	72 hrs
T. minuta Leaf oil 3%	63.775[a]	68.75[c]	84.500[b]
T. minuta Leaf AqE 70g/L	37.725[b]	55.050[d]	76.000[bc]
A. indica seed oil 3%	71.125[a]	89.425[b]	94.950[a]
A. indica Leaf oil 3%	33.525[b]	47.000[e]	55.450[d]
A. indica Leaf AqE 70g/L	43.725[b]	56.700[d]	70.550[c]
Amitraz 20EC 60µL	72.000[a]	99.000[a]	100.000[a]
Control	12.000[c]	19.500[f]	19.500[e]
LSD	11.631	6.4403	9.1288
CV	10.61	4.50	5.55

Means followed by the same letter (s) with in a column not significantly different from each other at 5%, LSD.

Leaf Bioassay Spray of TSSM Eggs

All the tested pesticides exhibited significant difference in causing mortality of TSSM eggs after 12 days of application (P<0.05). Amitraz 20 EC caused the highest percentage mortality of TSSM eggs (92.28%), followed by *T. minuta* (83.74%). The lowest percentage mortality of eggs was obtained by the application of *A. indica* leaf aqueous extracts (21.34%). The highest hatching of TSSM eggs to offspring was recorded from *A. indica* leaf aqueous extracts (78.66%), followed by untreated control (91.5%) (Table 4).

Table 4. Mean percent mortality of two-spotted spider mite (TSSM) eggs twelve days after treatment application

Treatments	Mean percent
Amitraz 20EC	92.28±[a]
T. minuta leaf oil 3%	83.74[b]
T. minuta leaf AqE 70g/L	58.44[c]
A. indica seed oil 3%	48.63[d]
A. indica leaf oil 3%	42.81[d]
A. indica leaf AqE 70g/L	21.34[e]
Control	8.5[f]
Cv	7.15
LSD	8.36

Means followed by the same letter (s) with in a column not significantly different from each other at 5%, LSD.

DISCUSSION

The leaf disc spraying Laboratory experiment showed that *A. indica* seed oil and *T. minuta* leaf oil 3% had comparable miticidal effect with Amitraz 20EC (standard check) throughout the observation period. On the other hand the leaf disc dipping experiment result indicated that *A. indica* seed oil, *T. minuta* Leaf AqE 70g/L *and A. indica* Leaf AqE 70g were the most promising botanical miticides under laboratory conditions almost throughout the observation period. In other words, both the leaf disc spraying and dipping methods gave almost similar efficacy to Amitraz 20EC or the standard check used in the experimentation. In line with this study, Cenusa *et al*. (2016) reported that *A. indica* seed oil reduced the number of red spider mites. The current finding also agreed with the finding of Mmbone *et al*. (2014) and Premalatha *et al*. (2018), who reported that the acaricidal activity of aqueous extracts of *T. minuta* and *T. tenuifolia* was effective against TSSM in the laboratory. They also added that *T. minuta* leaf extract caused 55% mortality of *T. urticae* within 24 hours of application (Mmbone *et al*., 2014).

This finding also revealed that Amitraz 20 EC is more toxic to TSSM eggs than TSSM adults, followed by *A. indica* seed oil and *T. minuta* leaf oil after 72 hours of application, while *A. indica* leaf AqE was the least effective. But only *T. minuta* leaf oil is as effective as the standard check (Amitraz 20 EC) against two spotted spider mite eggs though there was statistically significant difference in efficacy between the two. In agreement with this finding, Brito *et al.* (2006) also reported that *A. indica* extracts (neemseto) significantly reduced *T. urticae* adults and eggs at higher concentration rates than *p. persimilis* at lower doses. Their finding also confirmed that even at lower doses, *A. indica* extract was more effective than the two spider mite predator (*P. persimils)* on TSSM. *A. indica* oil caused 100% mortality of young females in the laboratory (Tsolakis *et al*., 2002).

The aqueous extracts of *A. indica* had a lower effect on TSSM eggs than aqueous extracts of *T. minuta* in the present result. There is scarcity of information on the botanicals to verify the current work. The current study agreed with the report of Bhuyan *et al*. (2017) who stated that Azadirachtin 5% suppresses hatchability by 70.00%. In contrast to this finding and others discussed above, Martínez-Villar *et al*. (2005) reported that Azadirachtin had no effect on fertility and offspring development of TSSM. Sarmah (2016) also reported that *A. indica* extract had ovicidal activity at higher concentrations than lower (e.g. 25% at 5, 56.67% at 8 and 65% at 10% concentration). Mourao *et al*. (2004) indicated that a significant reduction of TSSM egg (25%) was obtained at 4% and above concentrations of *A. indica* kernel extract.

In summary, it was found that the extracts of *A. indica* and *T. minuta* were effective which is in line with a number of authors including Phoofolo *et al*. (2013) who reported the effectiveness of the extracts of the botanicals against mites, whiteflies, aphids, thrips, mealybugs and scale insects. It killed larvae of the sugar beetroot maggots, *Tetanopsmyopae formis*, at higher concentrations (0.75%) and prevent pupation at lower (0.5%) concentrations (Dunkel *et al*., 2010). Its effect was also reported on stored product beetles (Keita *et al*., 2000), mosquitoes (Basabose *et al*., 1997) and armyworms. *T. minuta* is used to repel insect pests like aphids when intercropped with cabbage plants compared with mono-cropping (Jankowska *et al*., 2009). The efficiency of plant extracts for the control of mites is considered satisfactory when mortality rates are higher than 60% and excellent when they exceed 80% (Potenza *et al.,* 2006). Therefore, *A. indica* seed and *T. minuta* leaf oil extract were satisfactory in TSSM adult control in the present experiment with average percentage mortality of greater than 78% after 72 hours of applications in both leaf dip and spray bioassay methods. This showed that, if similar results could be obtained under field condition, these botanical acaricides could be considered as a component of integrated management of TSSM. Natural products contain compounds that degrade more rapidly than most synthetic chemicals, signifying a decrease in the chance of pest arthropods developing resistance, minor residual effects, and low toxicity to humans (Veronez *et al*., 2012).

CONCLUSIONS

Both leaf disc spray and leaf dip application methods of *A. indica* seed oil, *T. minuta* Leaf AqE 70g/L. *and A. indica* Leaf AqE 70g were promising botanical miticides under laboratory conditions almost throughout the observation period. *A. indica* seed oil and *T. minuta* leaf oil 3% had comparable miticidal effect with Amitraz 20 EC. Amitraz 20 EC was superior to almost all the botanical miticides on the eggs of the two spotted spider mite. If similar effect could be obtained under field conditions, these miticides can replace Amitraz 20 EC or be used in alternate application in the integrated management of the pest to delay pest resistance, reduce cost of production and minimize environmental backlash. This research finding, therefore, indicated another management option of TSSM for subsistence farmers who suffer losses from the pest. Alternate applications can also increase the efficiency of integrated management as the synthetic chemical had superior efficacy against the two spotted spider mite eggs. Further investigation is suggested on Amitraz 20 EC, *A. indica* seed oil and *T. minuta* leaf oil alone, in combination or in alteration under variable field conditions to develop sound management option against the pest.

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