Egypt. J. Plant Prot. Res. Inst. (2021), 4 (4): 612 –622

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Impact of some plant oils and hexaflumuron against of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) and *Tetranychus urticae* (Acari: Tetranychidae) on cotton plants

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ARTICLE INFO

Article History

Received:25/10 / 2021

Accepted:15/12 /2021

Keywords

Cotton , plant oils , hexaflumuron *Phenacoccus solenopsis* and *Tetranychus urticae.*

Abstract:

The cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) and the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) were predicted to become two of the most damage cotton pests within the next few years and polyphagous and live to gather. Field trials were carried out during 2020 and 2021 seasons at, Sakha Agricultural Research Station, Kafr El-Sheikh Governorate to the efficacy of garlic, moringa and jojoba essential oil and hexaflumuron (Scorch) (IGR) were tested against their toxicity to the immature stages and adult for both pests as well as biochemical studies. Plant essential oil and insecticide are commonly used to control *P. solenopsis* and *T. urticae* after 3, 7, 10 and 13 days post treatment, the average percentage of reduction will be calculated. The findings showed that the average % of reduction of the mealybug was 50.49, 38.44, 23.65 and 22.29 % by hexaflumuron, garlic, jojoba and moringa in the first season, respectively. While the second season was 51.98, 42.13, 27.24 and.24.76 with *P. solenopsis*, respectively. Thus, the trials indicated that the reduction % of *T. urticae* were 53.40, 39.44, 24.86 and 24.65 in the first season and 59.22, 50.13, 23.01 and 22.86 in the second season as well, respectively. The impacts of hexaflumuron, garlic, moringa and jojoba on trehalase, chitinase and protease activities were tested against the 3rd instars nymphs of the mealybug and all stages of the mite. The difference in trehalase and protease activities were insignificant between garlic and control after 3 days, but decreased significantly after 10 days of treatment for both pests, while hexaflumuron increase significantly between moringa, jojoba and control after 7 and 10 days after treatment. After 3 and 7 days of treatment, the difference in chitinase activity was significant between garlic and control. The data in the first season and the second season showed that, hexaflumuron was the most effective compound in reducing the population density of motile stages of *T. urticae* followed by garlic oil, whereas moringa oil and jojoba oil were the least effective compounds in reducing the population density of motile stages of *T. urticae* in the field. The pests, four toxic materials, garlic, moringa, jojoba and hexaflumuron in all experiments can be useful for developing safe elements for an integrated pest management strategy.

Introduction

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) and the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) are considered the most recent invasive sap suckling pests in Egypt and many parts of the world attacking the cotton plantations and many other agricultural crops of economic importance (Fuchs *et al*., 1991 and Ibrahim *et al*., 2015) as well as the cotton is considered of the most preferred hosts of the mealybug and mite. Causes direct damages by sucking the cell sap and becoming stunted, weak and fruit a few small bolls resulting in severe economic yield losses (Dhawan *et al*., 2007).

P. solenopsis infestation on many other field crops could be effective, control using plant extract, mineral oils and insecticides (Suresh *et al*., 2010; Kumar *et al*., 2012 and El-Zahi *et al*., 2016). The mealybug feeds on all the green parts of the infested plants which become stunted and it is predicted to become one of the most dangerous economic pests during the next years in Egypt where the agroecosystem is close adequate to its development and spread (El-Zahi and Farag, 2017). The use of essential oil and plant extract in pest control could be an effective alternative compared to pesticides. In Egypt, toxicity of chlorpyrifos, biochemical, IGR and Jojoba oil against 2nd and 4th instar of *Spodoptera littoralis* (Boisduval) (Lepidoptera ; Noctuidae) and their effect on some biological characters as well as fecundity were studied on 4th instar larvae (Gaaboub *et al*., 2012). Dimetry *et al*. (2017) tested moringa trees that decreased the weight gain significantly in the treated larvae of *S. littoralis* in comparison with the control. The cotton mealybug and the two spotted spider mite are a polyphagous pest, feeding on a wide variety of plants. The host range of this mealybug includes okra, eggplant, and cotton (El-Fakharany, 2020 and El-Zahi and Farag, 2017).

P. solenopsis infestations on different hosts could be effectively controlled using synthetic insecticides, plant extracts, mineral oils, and biological control agents (El-Zahi *et al*., 2016). Today, among the first observations carried out in this field, two were particularly focused on the effect of essential oils on insect pests. Essential oils are presently regarded as a new class of ecological products for controlling insect pests. The diversification of the approaches inherent in IPM is necessary for better environmental protection, the chemical complexity of essential oils, several mechanisms being contingent on the phytochemical pattern of the oils and the sensitivity of the insect species could be involved. Jojoba oil is suggested as a safe product with a potential for use as a biocide in IPM especially in urban countries where the use of chemical insecticides is discoursed (Abdel-Razik and Mahmoud, 2017). Spider mites feed by inserting their needle-like piercing-sucking mouthparts inside the plant tissue and they prefer to feed on the lower leaf surface (Attia *et al*., 2013). Natural plant extracts are causing damage of economic importance in agriculture. Plant essential oils have more toxic effects on pests through contact, ingestion and fumigation; so, plant essential oils or their constituents have significant behavioral effects on spider mites, especially as *T. urticae* (Akhtar *et. al*., 2010).

The aim of the present work is to study the efficacy of garlic, moringa and jojoba essential oil and hexaflumuron (Scorch) (IGR) against the immature stages and adults for *P.*

solenopsis and *T. urticae* as well as biochemical studies.

Materials and methods

1. Plant oils and insecticidal:

Commercial formulations of hexaflumuron (Scorch 10 % EC) (IGR), (Syngenta Agrosciencs, Swizerland) and three plant oils, garlic (*Allium sativum* L.), moringa (*Moringa oleifera*) and jojoba (*Simmondsia chinensis*) produced by Egyptian Natural Oil Co. used at the rate of 1.5 liter/F. They were dissolved in water containing 1% Triton, were tested for their toxicity to *P. solenopsis* and *T. urticae* under field conditions.

2. Field evaluation of *Phenacoccus solenopsis* and *Tetranychus urticae*:

The field experiment was conducted during the summer of 2020 and 2021 to evaluate the efficacy of one insecticide and three plant oils against *P. solenopsis* and *T. urticae* on the previously mentioned cotton plant variety at the farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt. The experiment was laid out in a randomized block design with five treatments. Each treatment contained three replications in an area of approximately 1500 m^2. Five plots were allocated randomly to each treatment. During the two seasons, these experiments did receive plant oil and insecticide treatments, the spraying was done 8-11 am. A knapsack sprayer provided with one nozzle delivering 1.5 letters/feddan was used to plant oils and hexaflumuron (Scorh 10% EC by rate 200 cm/ Fedden. The micron Ulva (ULVAT), Nozzle: Red nozzle to treatment Ec. According to the method described by Ahmad *et al*. (2011). Ten apical twigs of the same age from the cotton plants were randomly selected and labeled appropriately for further observation from each replicate to count all stages of *P. solenopsis*. Treatments were imposed when an enough mealybug population and mite were observed in the experimental block. Samples of 10 cotton leaves were randomly collected from each plot before and after treatment of the two spotted spider mites. Observations were recorded a day before spray and 3, 7, 10 and 13 days after spray. The percentage reduction of infestation was calculated for each treatment, according to Handerson and Tilton (1955) and Duncan (1955) multiple range tests at the 5% level was used for statistical analysis of significant differences among treatment.

% redaction= {1-(NCBS/NCASXNTAS/NTBS)}X100

NCBS= No. in cont. before spraying
NCAS= No. in cont. after spraying
NTAS= No. in treat. after spraying
NTBS= No. in treat. before spraying

3. Biochemical studies:

All the biochemical analysis was carried out in the Faculty of Agriculture, Kafr El-Sheikh University. Observations were recorded enzyme activities after spray on three, seven, and ten days. Total body tissue samples were collected from individuals treated is fed on treating leaves. Pests bodies treated or untreated were homogenized in distilled water (One gm. insect bodies/5ml) using a chilled glass teflon tissue grinder for 3 min. Homogenates were centrifuged at 8000 r.p.m. for 15 min at -2^{o}C in a refrigerated centrifuge. The supernatant used was stored at -5^{o}C until the use (Max-2 week) for determination of some enzyme activities included trehalas, chitinase, and protease (Figure 1).

4. Statistical analysis:

The collected data were subjected to one-way analysis of variance (ANOVA), and the means separated using Duncan's (1955) Multiple Range Test at $P < 0.05$ using SAS program (SAS, 1995).

Figure (1): Preparation of *Phenacoccus solenopsis* and *Tetranychus urticae* for enzymes analysis.

Results and discussion

1. Impact of some plant oils and hexaflumuron on *Phenacoccus solenopsis* and *Tetranychus urticae* under field conditions:

The first incidence of *P. solenopsis* and *T. urticae* was recorded in June, the highest population density was noticed in September. Also, in both seasons the infestation started when the cotton plants aged were three months. The effect of garlic, moringa, jojoba oils and hexaflumuron were tested under field conditions against all immature stages and adults of the mealybug infesting cotton plants during 2020 and 2021 by using ULVA+ spraying equipment after 3, 7, 10 and 13 days post treatment (Table 1). Results showed that there is no mortality in the check (Untreated from all experiment day). Results indicated that the average numbers percentage of *P. solenopsis* started at least after treatment and the population decreased gradually and its least number, were 91.47, 78.37, 60.93 and 53.93 numbers with hexaflumuron after 3, 7, 10 and 13 days post treatment, respectively. While in case of garlic showed the average numbers percentage value (102.36, 98.16, 81.47 and 71.28 numbers after 3, 7, 10 and 13

days post treatment, respectively). While jojoba and moringa were less toxic, recording 178.63, 191.38, 146.79 and 134.52 numbers with Jojoba and 166.52, 175.42, 143.38 and 211.73 numbers on moringa after 3, 7, 10 and 13 days, respectively in the first season. However, in the second season (2021), the data cleared that hexaflumuron proved to be the most effective against the mealybug, while garlic and jojoba showed effectiveness among them, indicating that the numbers % of *P. solenopsis* on cotton plants were 87.63, 75.45, 52.85 and 48.55 numbers in hexaflumuron after 3, 7, 10 and 13 days, respectively compared to the control. Whereas jojoba recorded the least mean numbers 167.58, 184.58, 130.45 and 123.63 numbers after 3, 7, 10 and 13 days post treatment, respectively. The results are shown in Table (2) indicated that hexaflumuron and garlic caused the highest reduction in mealybug (50.49 and 38.44 %, respectively) followed by jojoba and moringa (23.65 and 22.29% respectively), in the first season. While hexaflumuron and garlic in the second season (2021) had a similar effect on mealybug (51.98 and 42.13% reduction, respectively), followed by jojoba and moringa (27.24 and 24.76%, respectively).

The present results are in parallel with El-Zahi and Farag (2017) and Sahito *et al*. (2011) who observed the highest infestation of *P. solenopsis* on cotton during September and October. Soltan (2020) cleared that the mortality percentages of nymph instars of grasshoppers were jojoba 96%, moringa 65% and cascade 87% after 12 days post treatment, while Abd El-Rahman (2003) indicated that jojoba caused 83.8 and 90.8% mortality against *Liriomyz atrifolii*. (Burgess) (Diptera: Agromyzidae) larvae at 0.5 and 1% respectively. The present results in this concern, agreed with Gaaboub *et al.* (2012) who observed the toxicity of IGR (Lufenuron) and jojoba oil against 2nd and 4th of instar larvae *S. littoralis* and their effect on some biological characters and fecundity. jojoba, moringa and cascade against nymph instars of the grasshoppers, after 2, 4, 6, 8, 10 and 12 days post treatment in the field.

Salem *et al*. (2003) revealed that jojoba oil formulation was the potent agent against both whitefly and leafhopper species where the LC50 was 5.4% for *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and 6.4% for *Empoasca decipiens* Paoli (Hemiptera - Cicadellidae), respectively. Abdel-Razik and Mahmoud (2017) showed that 2nd or 4th instar larvae of cotton leafworm, *S. littoralis* exposed to jojoba extract for 24 hrs were greatly suffered from toxic effects which give good evidence for using jojoba as an element for the integrated management of insects. The highest mortality percentage (100%) of *Schistocerca gregaria* nymphs was calculated at 10% jojoba oil (Halawa *et al.,* 2007). Buprofezine was the least toxic one with LC_{50} value of 121.79 and 146.14 mg AI L-1 on eggplant and okra. In an attempt to control this pest, buprofezin, was tested for its influence against *P. solenopsis* on eggplant and okra under field conditions (El-Fakharany, 2020). Rizvi *et al.* (2015) found that spirotetramat proved significantly superior in controlling *P. solenopsis*. El-Zahi *et al.* (2016) found that imidacloprid and thiamethoxam showed the highest efficacy against *P. solenopsis* recording 89.2 and 84.6% reduction of the insect population while emamectin benzoate failed to exhibit sufficient *P. solenopsis* control. Unfortunately, recent studies reported that *P. solenopsis* has developed resistance to spirotetramat (Ejaz and Ali Shad, 2017 and Rezk *et al.,* 2019).

The data in the first season presented in Tables (3 and 4) had a similar effect on mite and showed that hexaflumuron was the most effective compound in reducing the population density of motile stages of mite, *T. urticae* (82.47, 67.55, 52.34 and 45.66 numbers after 3, 7, 10 and 13 days, respectively) with 53.40% reduction followed by garlic oil (90.53, 87.54, 73.33 and 66.23 numbers after 3, 7, 10 and 13 days, respectively) with a percentage reduction of 39.44%. Whereas moringa oil and jojoba oil were the least effective compounds in reducing the population density of motile stages of *T. urticae* (24.86% and 24.65%, respectively). But in the second season hexaflumuron was the most effective compound in reducing the population density of motile stages of mite, *T. urticae* (73.56, 62.66, 47.23 and 39.54 numbers after 3, 7, 10 and 13 days post treatment, respectively) with 59.22% of the reductions, followed by garlic oil was (50.13%). Whereas moringa oil and jojoba oil were the least effective compounds in reducing the population density of motile stages of *T. urticae* (23.01 and 22.86 %, respectively). In general, in all treatments the most effective compounds in reducing the population density. Based on these reductions, all compounds were effective in reducing the population density of motile stages of mite *T. urticae.*

Table (1): The tested compounds effect *Phenacoccus solenopsis* on cotton plants in field condition.

Compounds	No. of *Phenacoccus solenopsis* per-treatment	No. of *Phenacoccus solenopsis* post-treatment			
		3rd day	7th day	10th day	13thday
Season 2020					
Hexaflumuron	188.43	91.47	78.37	60.93	53.93
Garlic oil	186.61	102.36	98.16	81.47	71.28
Moringa oil	211.73	166.52	175.42	143.38	211.73
Jojoba oil	231.36	178.63	191.38	146.79	134.52
Control	262.73	177.91	185.48	216.72	258.81
Season 2021					
Hexaflumuron	185.77	87.63	75.45	52.85	48.55
Garlic oil	192.23	92.64	91.65	78.74	76.57
Moringa oil	201.84	153.53	170.73	132.13	120.64
Jojoba oil	220.54	167 .58	184.58	130.45	123.63
Control	265.92	173.57	182.63	225.65	248.74

Table (2): The reductions effect of tested compounds on motile stages of *Phenacoccus solenopsis* on cotton plant in field condition.

Compounds	% reduction				General mean
	3rd day	7th day	10th day	13th day	
Season 2020					
Hexaflumuron	28.64	41.36	60.87	71.09	50.49
Garlic oil	19.36	25.83	47.17	61.42	38.44
Moringa oil	15.61	16.81	18.06	38.71	22.29
Jojoba oil	13.49	16.63	23.22	41.27	23.65
Season 2021					
Hexaflumuron	27.82	42.73	66.71	72.29	51.98
Garlic oil	26.26	32.77	52.07	57.77	42.13
Moringa oil	16.37	19.26	23.40	36.64	24.76
Jojoba oil	16.25	18.00	30.79	40.57	27.24

Table (3): The tested compounds effect on motile stages of *Tetranychus urticae* on cotton plants in field condition.

Compounds	No. of *Tetranychus urticae* per-treatment	No. of *Tetranychus urticae* post-treatment			
		3rd day	7th day	10th day	13th day
Season 2020					
Hexaflumuron	172.76	82.47	67.55	52.34	45.66
Garlic oil	168.44	90.53	87.54	73.33	66.23
moringa oil	198.56	152.45	166.13	130.16	106.28
Jojoba oil	201.73	162.53	178.46	137.34	112.37
Control	256.77	180.33	182.27	212.53	246.76
Season 2021					
Hexaflumuron	178.54	73.56	62.66	47.23	39.54
Garlic oil	180.75	84.57	76.42	63.53	53.43
moringa oil	192.59	142.53	153.88	112.45	98.62
Jojoba oil	206.44	151.63	166.42	122.66	104.88
Control	260.64	175.44	178.73	197.49	242.79

Table (4): The reductions effect of tested compounds on motile stages of *Tetranychus urticae* on cotton plant in field condition.

Compounds	% reduction				General mean
	3rd day	7th day	10th day	13th day	
Season 2020					
Hexaflumuron	32.21	45.25	63.64	72.51	53.40
Garlic oil	23.68	27.24	47.75	59.10	39.44
Moringa oil	7.48	17.13	21.33	53.52	24.86
Jojoba oil	14.40	23.85	18.30	42.06	24.65
Season 2021					
Hexaflumuron	41.49	50.86	68.25	76.30	59.22
Garlic oil	33.56	40.80	57.82	68.37	50.13
Moringa oil	5.08	11.86	29.93	45.20	23.01
Jojoba oil	4.29	12.85	28.69	45.63	22.86

Asmae *et al*. (2019) investigate the toxicity of the essential oils of Salvia officinal and Eucalyptus globulus against the adults of two spotted spider mites, *T. urticae*. The results showed that the two oils increase spider mite mortality in adults. Gaber and Nasr (2020) investigated the effects of neem essential oil and aqueous neem extract on female adult *T. urticae*. The results showed that neem essential oil was more effective than aqueous neem extract.

2. Biochemical studies:

The data in Tables (5 and 6) showed that, the effect of garlic, moringa, jojoba and hexaflumuron on trehalase, chitinase and protease activities of the cotton mealybug and mite. Jojoba highly significant increased trehalase activity after 3, 7 and 10 days compared to control. The trehalase activity difference was significant increased with garlic after 7 and 10 days and was insignificant after 3 days, while causing highly significant increased after 3 days with hexaflumuron and significant after 7 and 10 days from treatment compared to control of *P. solenopsis* and *T. urticae*.

On the other hand, garlic showed highly, significant increases in chitinase activity after 3 days, but was significant after 7 and 10 days compared with control with both pests moringa illustrated that insignificant after 3, 7 and 10 days compared with control. Jojoba increased the chitinase activity significantly after 3, 7 and 10 days compared with control of *P. solenopsis* and *T. urticae*.

Data presented showed the hexaflumuron increase highly significant on chitinase activity after 3, 7 and 10 days after treatment compared with control on both pests and while garlic showed the insignificant difference in activity in protease after 3 days but highly significant increased after 7 and 10 days compared with control. Moringa significant difference in activity in protease after 3 and 7 days. Also, jojoba induced a highly significant increase compared with control after 7 and 10 days. While hexaflumuron increased protease activity highly significantly after 3 days from treatment compared with control all this data on *P. solenopsis*. But with *T. urticae*. was significant compared with control.

Table (5): The effect of the plant oils and hexaflumuron on enzymes activity of *Phenacoccus solenopsis.*

Enzymes	Days after Treatment	Plant oils			Hexaflumuron	Control	LCD
		Garli	Moringa	Jojoba			
Trehalase Mgmol /min. /mg protein	3	395^{c}	426b	582^{a}	551^{a}	370c	63.27
	7	401^{c}	^{b}415	435^{a}	418^{b}	294^{d}	66.21
	10	345^{c}	340^{b}	407^{a}	315^{c}	282^{d}	73.44
Chitinase Mgmol /min. /mg protein	3	510^{a}	400c	483^{b}	525^{a}	420^{c}	42.33
	7	410c	381^{d}	815^{b}	1128^{a}	311^{d}	512.11
	10	346c	281^{d}	933^{b}	1677^{a}	291^{c}	589.11
Protease Mgmol /min. /mg protein	3	4.41c	5.21^{b}	6.11^{a}	6.32^{a}	4.21^{c}	3.89
	7	15.32a	10.23^{b}	15.82^{a}	10.30^{b}	5.52^{c}	4.11
	10	17.65a	14.11^{c}	18.31^{b}	13.84^{c}	8.24^{d}	5.57

Table (6): The effect of the plant oils and hexaflumuron on enzymes activity of *Tetranychus urticae*

Enzymes	Days after treatment	Plant oils			Hexaflumuron	Control	LCD
		Gar	Moringa	Jojoba			
Trehalase Mgmol /min. /mg protein	3	289^{c}	376^{b}	423^{a}	405^{a}	366^{c}	58.50
	7	316^{c}	366^{b}	377^{a}	366^{b}	285^{d}	61.25
	10	233^{c}	235^{c}	357^{a}	272^{b}	273^{b}	67.55
Chitinase Mgmol min. /mg protein	3	457^{a}	312^{c}	338^{c}	390^{b}	416^{a}	37.47
	7	355^{c}	268^{d}	634^{b}	917^{a}	318^{d}	358.32
	10	235^{c}	164^{d}	748^{b}	1240^{a}	288^{c}	527.16
Protease Mgmol /min./mg protein	3	3.55^{c}	3.80	4.88^{a}	5.35^{a}	4.33^{b}	2.73
	7	10.24^{a}	6.94^{c}	8.45^{c}	9.55^{b}	5.49^{d}	3.81
	10	13.84^{a}	10.54^{c}	13.35^{b}	13.14^{a}	9.12^{d}	4.63

These results agree with Soltan (2020) found that no significant difference activity between moringa and control after 2 days, the difference in chitinase and protease in activity was insignificant between moringa and control after 2 and 4 days. The difference in protease activity was insignificant between moringa and control after 2 days while causing an increase significant between jojoba and control after 4 and 6 days after treatment. Soltan (2014) observed that the difference trehalase activity of desert locust was insignificant between garlic and control after 2 days while increased significantly after 4 and 6 days post treatment. The molting fluid contains protease and chitinase, enzymes that digest the main constitution of the old end cuticle (Reynolds and Samuels, 1996). Accordingly, mortality percentage and changes in enzymes activities of the insects were greatly affected. Thus, it could be concluded that garlic oil, moringa, jojoba and hexaflumuron could be used as an effective natural product to be included in the integrated pest management program of the cotton mealybug in the field, Tanani *et al.* (2012) found that the treatment of *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) by tebufenozide caused a significant increase in trehalase activity after 4 days from treatment.

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