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Laboratory and field evaluation of environmentally safe chemicals against the two-spotted spider mite *Tetranychus urticae* (Koch) and its predatory mite *Amblyseius gossipi* (El-Badry)

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ABSTRACT

Laboratory and field experiments were carried out to evaluate the toxic effect of five compounds; three hydrocarbon oils (KZ-oil, chemesol and supermasrona), one pyrethroid (cypermethrin) and one biocide (Biofly) against adult female of the two spotted spider mite, *Tetranychus urticae* (Koch) and adult female of predatory mite, *Amblyseius gossipi* (El-Badry). The effect of sublethal doses of these compounds on some biological and behavioural characteristics of this mite and its predator was also examined. The evaluation of these compounds against *T. urticae* on soybean plants in the field was also studied. Cypermethrin can not be recommended when safety and selectivity index are taken in consideration, but its toxic effect to prey mite and its low mammalian toxicity make us to keep it in mind under certain conditions. It was one of the most effective compounds on prey egg consumption by the predator *A. gossipi*. The mineral oils are of special purpose in mite control for their physical effect and relative high safety index on spider and predator mites. Supermasrona is a promising oil in integrated mite control programmes. Biofly did not exhibit certain trend for its ovicidal effect on different egg stages and its effect on prey egg deposition was decreased as time passing after treatment increased. Also, Biofly was one of the most safe compounds that allowed the predator to consume the contaminated prey eggs. In general, the time elapsed before introducing the contaminated prey eggs to adult predators is partially responsible for prey egg consumption and, consequently, the predator egg production. From the field trials, the mineral oils when used on soybean plants exhibited high mean reduction in motile and egg stages while cypermethrin was the least effective.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* is considered one of the major pests in Egypt attacking different agricultural crops such as field crops, vegetables, fruits and ornamental plants. The infestation by mite caused a great damage to these infested plants followed by a secondary infestation by various pathogens such as virus, bacteria and fungi.

The spider mite problem was not solved by reliance solely of the use of chemicals of an acaricidal effect since mites very rapidly developed resistance to many of these compounds (Huffaker *et al*., 1970). Alternative non chemical methods of control have been widely assessed, the most successful one had been the exploitation of predatory mites (Van De Vrie *et al*., 1972), which are commonly found in agricultural ecosystems and in unsprayed orchards.

The use of predators had proved to be the most effective control method for tetranychid mites and the most effective predators have been found in the family Phytoseiidae (Abou Awad and El-Banhawy, 1985). *Amblyseius gossipi* (El-Badry) has one of the fastest development rates and has high oviposition and predation rates. It was collected and described by El-Badry (1967).

The possibility of controlling phytophagous mites by a combination of biological and chemical methods had proved to be a less costly and more permanent method of control than pesticides alone (Croft, 1978 and Hislop and Prokopy, 1981).

Petroleum oils were used because they are cheaper and more safe to human and environment. El-Sisi and El-Hariry (1989) and El-Hariry *et al*.(1998) used many types of petroleum oil for controlling mites. Those local, safe and cheap materials gave good effects against some pests which attack economic crops in Egypt. Clearly, if chemical, microbial pesticides and biological methods are successfully integrated, then the impact of pesticides used to control key pests and diseases must be minimized while the beneficial arthropods must be introduced.

The present study was carried out to examine the toxic effect of three hydrocarbon oils, one biocide (Biofly) and one pyrethroid (cypermethrin) against adult female of the two-spotted spider mite *T. urticae* and its predatory mite, *A. gossipi*; and the side effect of sublethal doses of tested compounds on some biological aspects of spider mite, *T. urticae* and its predatory mite, *A. gossipi*. Field evaluation of these compounds on spider mite, *T. urticae* on soybean plants was also studied.

MATERIALS AND METHODS

A. Laboratory studies

Prey cultures: Two-spotted spider mite, *T. urticae* (Koch) (Acarina: Tetranychidae) colonies were obtained from castor bean plants from Kafr El-Sheikh Governorate and reared under laboratory conditions according to Dittrich (1962).

Predator culture: The predator *A. gossipi* (El-Badry) was reared on pollen grains of castor oil (*Ricinus communis*) plants according to the technique used by Overmeer *et al.*(1982).

Chemical used: Five compounds were used: cypermethrin: [(RS)-α-cyano-3-phenyloxy-benzyl (IRS, 3RS; IRS, 3RS)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] as 25% EC, Biofly: [a trade name of the entomopathogenic fungus, *Beauvaria bassiana* (Balasamo) as a liquid containing $3x10^7$ conidia/ml], KZ-oil: [formulated mineral oil as 95% EC], chemesol: [formulated mineral oil as 95% EC] and supermasrona [formulated mineral oil as 94% EC].

Experimental techniques

1. Toxicity of tested compounds to adult female mites *T. urticae* and its predator, *A. gossipi*: The toxic effect of tested compounds to the adult female mites, *T. urticae* and its predator, *A. gossipi* were evaluated by the leaf disc dip technique according to Siegler (1947). Mortality counts were made 24 hours after treatment. Correction for the control mortality was made by using Abbott's formula (1925).

2. Effect of compound residues on *T. urticae* egg laying and its hatchability: The residual effect of each tested chemical at LC_{25} level on adult prey mites was evaluated according to Keratum *et al.*(1994).

3. Effect of different compounds on hatchability of *T. urticae* eggs at different ages: The effectiveness of the tested compounds on *T. urticae* eggs of different ages was examined using the method of Staal *et al.* (1975).

4. Effect of compound residues on egg consumption and egg laying and its hatchability by predatory mite, *A. gossipi:* The method which was adopted by Keratum *et al.*(1994) was used to evaluate the effect of tested compound residues on egg consumption and egg laying and its hatchability by the predatory mite, *A gossipi*

B. Field experiment: An experiment was carried out in the farm of Faculty of Agriculture, Kafr El-Sheikh, Egypt to evaluate the efficiency of the tested compounds on spider mite, *T. urticae* infesting soybean plants variety "Crawford". Plots each of 1/200 feddan were used. Completely randomized block design was employed and four replicates were assigned for each treatment. All tested compounds were applied at half their recommended rates using knapsack sprayer with one nozzle. The rate of water used for diluting compounds was 200 liters/feddan. Samples of 10 soybean leaves were randomly collected from each plot before and after treatment at intervals of 2 days and one week later. The percentage reduction of infestation was calculated for each treatment according to Handerson and Tilton equation (1955):

$$\% \text{ mortality} = \left[1 - \left(\frac{\text{population in the control before spraying}}{\text{population in the control after spraying}}\right) \text{x} \left(\frac{\text{population in the treatment after spraying}}{\text{population in the treatment before spraying}}\right)\right] \text{x } 100$$

Duncan's multiple range test at the 5% level was used for statistical analysis of significant differences among treatments.

Equations:

1. Abbott's formula (1925): was used to correct % mortality according to natural mortality:

$$\text{Mortality } (\%) = \frac{\text{Mortality \% of treatment - mortality \% of control}}{\text{100 - mortality \% of control}} \text{ x } 100$$

2. Selectivity ratio of tested compound on predator mite *A. gossipi* was determined as follows according to Wilkinson (1976):

$$\text{Selectivity ratio (S.R.)} = \frac{LC_{50} \text{ of the compound on predator}}{LC_{50} \text{ of the compound on prey}}$$

3. $$\text{Selectivity index} = \frac{\text{S.R. of the tested compound}}{\text{S.R. of the most selective compound}}$$
(compound of the highest S.R. value)

4. Toxicity index of tested compound was determined according to Sun (1950) as follows:

$$\text{Toxicity index} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the tested compound}} \times 100$$

5. Safety index of tested compound on predator mite was determined according to Aref (1997) as follows:

$$\text{Safety index} = \frac{LC_{50} \text{ of tested compound on predator}}{LC_{50} \text{ of the least effective compound on predator}} \times 100$$

RESULTS AND DISCUSSION

A. Laboratory studies

Toxicity of some mineral oils, cypermethrin and Biofly to adult female mites, *T. urticae* (Koch) and its predator, *A. gossipi* (El-Badry)

Cypermethrin was used representing pyrethroids in this study. It was the most toxic material (LC_{50} = 265 ppm) comparing with the mineral oils (Table 1), while chemesol was the least toxic one. Hurkova (1984) found that cypermethrin at low concentration caused a knock down to *T. urticae* and the higher concentration (0.1%) had a repellent effect. Whereas Kovach and Gorsuch (1986) indicated that this compound (3.9 g a.i/100 L) caused the highest mortality of *T. urticae*. Cypermethrin is known to be of moderate toxicity to spider mite, in general, but the low mammalian toxicity and its environmental safety promote the awareness to be included in integrated pest management programmes. The pyrethroids were found sometimes to be of limited interest because of their secondary effect, especially on predator mite. It was the most toxic in the present study (LC_{50} = 20 ppm) to the adult predator, *A. gossipi*. These products will be in compatible to the biological control of phytophagous mites unless strains of these predators can be resistant to pyrethroids.

The three mineral oils used in this study (Table 1) were of the second category after cypermethrin in their toxicity to *T. urticae*. Supermasrona as the most toxic oil was about two fold as KZ-oil and about four fold as chemesol. In general, mineral oils are known to be physically effective on the different mite stages. Their effects depend on various factors. These hydrocarbon oils are used as insecticides, ovicides, herbicides and as carriers of oil-soluble pesticides emulsions. The hydrocarbon oils that are used for selective pest control on foliage of low chemical reactivity and known to be of the least toxic effect.

As reported by Deong *et al.*(1927), the highly refined spray of oil may be used as selective herbicide and may be responsible for suffocation of the insect or mite individual. The mineral oils were evaluated against the different stages of spider mite and were indicated to be successful (Nassef, 1998; Rizk *et al.*, 1999 and Gamieh *et al.*, 2000).

When the three oils were tested against *A. gossipi*, KZ-oil and chemesol were of moderate effect (LC_{50} = 800 ppm for each), while supermasrona was the least toxic. The toxicity parameters, safety index and selectivity index showed that supermasrona equal 100 for each. The last parameter is considered the most precise value that indicates how the compound behaves toward the two adult species of mites (prey and predator). In other words, the most safe compound against the predator and in the same time the most toxic to the prey mite is the most suitable compound that must be advised to involve in integrated pest management.

Table (1): Toxicity of some mineral oils, cypermethrin and Biofly to adult female mites *T. urtica* (Koch) and its predator *A. gossipi* (El-Badry).

Compound	*T. urticae*				*A. gossipi*				Toxicity parameters		
	LC_{50}* (ppm)	C.L.**	Slope Value	Toxicity Index	LC_{50} (ppm)	C.L.	Slope Value	Toxicity Index	Safety index	Selectivity ratio (S.R.)	Selectivity index
KZ-oil	1700	1164.4-2482.0	1.55	15.59	800	655.7-976.0	3.52	2.50	50	0.470	27.33
Chemesol	3850	3031.5-4889.5	2.11	6.88	800	575.5-1108.8	1.54	2.50	50	0.210	12.21
Supermasrona	930	744.0-1162.5	2.60	28.50	1600	1311.5-1943.4	2.59	1.25	100	1.720	100
Cypermethrin	265	210.3-333.9	1.97	100	20	14.3-28.0	1.74	100	1.25	0.076	4.42
Biofly*	66000	55462.2-78540.0	2.00	-	84000	60869.6-115987.6	1.81	-	-	1.270	-

* LC_{50} was determined as conidia/ml.
** C.L. : Confidence limits.

The microbial pesticide Biofly (*B. bassiana*), when compared in its toxicity against the phytophagous mite and its predator, was found to be of slight selective effect on predator mite (selectivity ratio 1.27). This result is in agreement with those reported by Karadzhov (1973), Bartkowski *et al.*(1988) and Derbalah (1999).

Effect of compound residues on prey egg deposition and its hatchability

The effect of sublethal concentrations of tested compounds (LC_{25}) on eggs deposited by adult female, *T. urticae* and their hatchability was studied. The data in Table (2) show that KZ-oil and cypermethrin caused the highest reduction in egg deposition compared to control all over the different times between treatment and deposition (zero-72 hrs), while supermasrona showed the least effect on egg deposition. In general, the effect of different compounds can be arranged descendingly as follows: KZ-oil > cypermethrin > chemesol > Biofly > supermasrona > control. The data, in general, indicate that the eggs deposited by adult *T. urticae* increased as the pesticide residue decreased (from zero time to 72 hrs) on the leaf discs. The number of eggs deposited on discs at zero time after treatment was less, in general, than that deposited at 24 hrs. Stafford and Fukushima (1970) found the same result with benomyl on *T. pacificus*.

Table (2): Egg deposited by adult female mite *T. urticae* during 3 days on different compounds - pretreated leaf discs and its hatchability.

Compound	No. of eggs deposited at indicated time after treatment				% Hatchability			
	zero time	24 hrs	48 hrs	72 hrs	zero time	24 hrs	48 hrs	72 hrs
KZ-oil	7	29	20	56	57.1	34.5	45.0	44.6
Chemesol	64	163	249	98	28.1	14.7	12.0	22.4
Supermasrona	408	621	548	477	58.1	60.1	70.6	55.6
Cypermethrin	12	55	35	59	16.7	40.1	45.8	17.0
Biofly	76	150	274	181	64.5	56.0	55.1	58.0
Control	550	550	550	550	91.8	91.8	91.8	91.8

There is no doubt that low levels of chemicals which do not cause mortality can influence this character. The present laboratory treatments simulate field conditions where the mites will be exposed to chemical residues on plant leaves through feeding on contaminated cell contents. The reduction in egg deposition may be due to interference with the division and growth of egg cells (Harries, 1961, 1963 and Nakashima and Croft, 1974). Stimulation of biochemical processes by small quantities of stressful chemical (hormoligosis) was suggested by Dittrich *et al.*(1974) as responsible for increase in egg laying of *T. urticae* exposed to carbaryl or DDT.

The data also indicate that KZ-oil, chemesol and cypermethrin were the most effective compounds on egg hatching, followed by supermasrona, but Biofly was the lowest in its effect on egg hatching.

Effect of different compounds on hatchability of prey eggs at different ages:

The ovicidal action of the tested materials on three different egg stages was carried out. The data in Table (3) exhibit that all compounds were highly toxic to eggs of *T. urticae* at its different stages (24, 48 and 72 hrs). In general, the effect of different compounds could be arranged descendingly as follows: chemesol = supermasrona > KZ-oil > cypermethrin > Biofly. Biofly had the lowest ovicidal effect on different egg stages. On the other hand, the three hydrocarbon oils exhibited about the same ovicidal action at different egg stages. No doubt that there is correlation between hatchability and egg age which may be negative or positive according to the physical and chemical properties of the tested compound. The chemicals of an ovicidal effect may act to prevent embryo formation or the compound may be of toxic effect to the developmental larval stages inside the egg membrane before hatching (El-Atrouzy *et al.*, 1989).

The nature of physical toxicity of the hydrocarbon oils make them safe compounds from the environmental point of view, and physical changes may be expected on egg surface due to the oil treatment and this is why there is no difference between different egg stages in their response to oil treatment.

Table (3): Effect of different compounds on hatchability of prey eggs at different stages.

Compound	% Hatchability of eggs at indicated stages		
	24 hrs	48 hrs	72 hrs
KZ-oil	1.2	0.8	13.8
Chemesol	1.9	1.9	zero
Supermasrona	2.8	10.6	zero
Cypermethrin	11.9	11.5	17.7
Biofly	11.8	1.4	19.8
Control	76.3	76.1	88.5

Effect of compound residues on feeding capacity and egg production of predatory mite, *A. gossipi*

From data shown in Table (4), the following points could be concluded:

1. The pyrethroid compound cypermethrin is the most effective on prey egg consumption and predator egg production.
2. Biofly and supermasrona are the most safe compounds that allowed the adult predator to consume the contaminated eggs.
3. The hatchability of predator eggs deposited on contaminated discs was highly affected by the hydrocarbon oil, chemesol.
4. The time elapsed before introducing the contaminated prey eggs to the predator is partially responsible for prey eggs consumption and accordingly the predator egg production.

It is interesting to know that the deposited eggs were not in correlation with the eaten ones. Mite activity can be influenced by the nature of the substrate of the surface (Blommers *et al.*, 1977 and Everson, 1979 & 1980). The activity pattern of *A. gossipi*, which was not measured in this study, may be responsible for the non-correlated relation between feeding capacity and oviposition in the predator. The presence of chemicals at low levels on the leaf surface may be irritant enough to make the predator in contact with the contaminated prey eggs and, accordingly, the consumed eggs seemed to be almost of the same level of untreated control.

Table (4): Prey egg consumption and egg deposition of predatory mite *A. gossipi* feeding on different compounds – pretreated prey eggs introduced at different times after treatment.

Compound	Time elapsed between treatment and egg introduction								
	Zero time			24 hrs			48 hrs		
	Average No. of consumed eggs/adult /day	Average deposited eggs/adult /day	Hatchability (%)	Average No. of consumed eggs/adult /day	Average deposited eggs/adult /day	Hatchability (%)	Average No. of consumed eggs/adult /day	Average deposited eggs/adult /day	Hatchability (%)
KZ-oil	20.00	0.50	76.0	16.00	0.25	100	13.13	0.00	0.0
Chemesol	10.38	0.50	0.0	15.00	0.38	34.0	26.38	0.38	0.0
Supermasrona	8.25	0.50	26.0	21.25	0.38	65.8	27.25	0.25	100
Cypermethrin	9.13	0.00	0.0	3.50	0.00	0.0	4.50	0.00	0.0
Biofly	13.13	0.25	0.0	26.38	0.50	100	18.63	0.38	100
Control	32.38	1.25	100	30.50	0.88	100	29.63	1.13	100

B. Field studies

Effect of different compounds on egg and motile stages of *T. urticae* on soybean plants in the field

The percentage reduction of infestation was calculated. The data presented in Table (5) show that the two oils supermasrona and KZ-oil were the most effective in reducing the population density of motile stages of *T. urticae* (95 and 91%, respectively), while cypermethrin exhibited the least reduction (62%). The same trend was almost shown in counts of the egg stage. Based on % reduction, all mineral oils were effective in reducing egg and motile stages. The biocide, Biofly did not show satisfactory control, cypermethrin was found to be the least effective compound in contrast to its results in the laboratory against motile and egg stage. These results are in accordance with that of Perring (1987) who found that cypermethrin was one of pyrethroids that caused significant increase in mite, *T. urticae* abundance. The same result was recorded by Bleicher *et al.*(1993).

The hydrocarbon oils exhibited efficiency in mite control. KZ-oil was found by El-Enany and Nawar (1988) to give 80% decrease in mite *T. urticae* population. KZ-oil and supermasrona gave more than 84% reduction in *T. arabicus* in the cotton fields (El-Zahi, 2000).

Clearly, if chemical, microbial pesticides and biological methods are successfully integrated, then the impact of compounds used to control key pests and disease must be minimized while the beneficial arthropods must be introduced.

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Table (5): Number of egg and motile stages of mite *T. urticae* on different compounds – treated soybean plants in the field.

Compound	No. of motile stages/ 40 leaves before treatment	No. of motile stages/40 leaves at indicated period after treatment		Mean reduction (%)	No. of eggs/40 leaves before treatment	No. of eggs/40 leaves at indicated period after treatment		Mean reduction (%)
		48 hrs	One week			48 hrs	One week	
KZ-oil	390	123	16	91.32	382	141	15	91.31
Chemesol	353	169	17	87.16	518	76	34	90.87
Supermasrona	320	53	15	94.89	371	77	34	87.12
Cypermethrin	255	308	95	61.96	318	207	117	57.01
Biofly	219	89	134	76.43	369	77	97	81.55
Control	182	369	411	-	401	419	623	-

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التقييم المعملي والحقلي للمركبات الآمنة بيئياً ضد الأكاروس النباتي (تترانيكس أورتيكا) والأكاروس المفترس (أمبليسيس جوسيباي)

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أجريــت التجارب المعملية والحقلية لتقييم التأثير السام لخمسة مركبات، ثلاثة زيوت معدنية هيدروكربونـــية ومبـــيد بيريـــثرويدي (ســـيبرميثرين) ومركب حيوي (بيوفلاي) ضد العنكبوت (تترانيكس أورتيكا) والأكاروس المفترس (أمبليسيس جوسيباي). كما تم اختبار التأثيرات الجانبية للجـرعات تحـت المميـــتة للمركبات السابقة على بعض الصفات البيولوجية للأكاروس النباتي والأكاروس المفترس. ودرس أيضاً تقييم المركبات السابقة ضد الأكاروس على نبات فول الصويا في الحقل.

أظهــرت النـــتائج أن مبـــيد الســـيبرميثرين لايمكن التوصية به إذا أخذت قيم فهرس الأمان والإختيارية في الإعتبار – ولكن ارتفاع سميته للأكاروس وانخفاض سميته للثدييات يجعلنا نضعه فـــي الأذهـــان تحت ظروف معينة. وأن الزيوت المعدنية ذات استعمال خاص لتأثيرها الطبيعي وارتفـــاع فهرس الأمان لها على الأكاروس ومفترسه. وأن زيت سوبرمصرونا مركب واعد في بـــرامج المكافحـــة المـــتكاملة. ولم يظهر المركب بيوفلاي تأثير محدد كمبيد بيض على أعماره المختلفة. كما أن تأثيره على وضع البيض كان ينخفض بمرور الزمن بعد المعاملة. كما أنه كان مـــن المركـــبات الأكثر أماناً بحيث يسمح لاستهلاك البيض الملوث بالمبيدات بواسطة المفترس. وبصـــفة عامة فإن الزمن المار قبل تقديم البيض الملوث بالمبيدات للمفترس يعتبر جزئياً مسئولاً عـــن اســـتهلاك البيـــض وبالتالي إنتاج بيض المفترس. ومن التجارب الحقلية اتضح أن الزيوت المعدنية المستخدمة على فول الصويا أظهرت انخفاضاً عالياً في اعداد الأطوار المتحركة والبيض بينما كان مركب سيبرميثرين الأقل تأثيراً.