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SIDE EFFECTS OF JUVENILE HORMONE MIMIC (JHM), PYRIPROXYFEN ON THE PREDATORY MITE, *PHYTOSEIUS FINITIMUS* (RIBAGA) (ACARI: PHYTOSEIIDAE)

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ABSTRACT: *Direct and indirect effects of Juvenile Hormone Mimic (JHM), Pyriproxyfen 10% EC on the predatory mite, Phytoseius finitimus (Ribaga) (Acari: Phytoseiidae) were studied under the laboratory conditions. The side effects of pyriproxyfen on mortality, developmental duration periods, life-cycle, male and female longevity, oviposition period, female fecundity and egg viability were evaluated. Results clearly indicated that pyriproxyfen exhibited no direct contact toxicity against mature and immature stages of the predatory mite, P. finitimus at all the tested concentrations (2.5 , 5 ,10 ppm).The highest concentration of 10 ppm showed low toxicity , causing 12.77 and 10.56 mortality % of protonymphs and adult females, respectively after 72 hours post treatment. In addition , pyriproxyfen exhibited no harmful indirect effects on developmental duration period, male and female longevity, female fecundity and egg viability at all the tested concentration. These results suggest that, pyriproxyfen could be a good alternative and interesting incorporation in IPM programs for controlling the two spotted spider mite, Tetranychus urticae due to its high toxicity on the target pest and has no harmful effects on it's predatory mite, P. finitimus, minimizing the usage of conventional chemical pesticides.*

Key words: *Pyriproxyfen, predatory mite, Phytoseius finitimus, side effects, biological aspects, toxicological effect.*

INTRODUCTION

The two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is a worldwide major pest species of agricultural crops (HO, 2000 &Takafuji *et al.,* 2000),it attacks most of vegetable, ornamental and field crops causing a severe damage in yield quality and quantity or even kills the host plants (Nachman & Zemek 2002).

Phytoseiidae mites are predators of phytophagous mites and some insects having an important role in the biological control of spider mites (Ali, 2006, Hagrass, *et al.,* 2008 and Azouz, *et al.,* 2011). The predatory mite, *Phytoseius finitimus* (Ribaga) (Acari: Phytoseiidae), is the most abundant natural enemy associated with *T.urticae* on cotton plants.

Broad-spectrum, nerve toxic pesticides such as organophosphate, carbamate, and pyrethroid may be have both directly and indirectly more harmful to natural enemies than non-nerve toxic type pesticides such as insect growth regulators (IGR's) (Ishaaya *et al*., 2007 and Cloyed, 2012). The systemic synthetic mimics of insect hormones, which are best known as insect growth regulators have been reported to be potent control agents against a number of pest insects of agricultural and fruit orchards (Fox, 1990). Pyriproxyfen 10% EC was designed to model on the natural juvenile hormone. Therefore, it has similar effects on the insect development and so called biorational pesticide. This compound is almost non-toxic to non-target organisms and natural enemies such as parasites,

predator insects and mites (Neumonn & Guyer, 1987).

Abdel- Hafez, *et al.,* 2014 studied the toxicological and biological effects of JHM, pyriproxyfen 10% EC against the two spotted spider mite, *T.urticae.* .Therefore, The present study aimed to evaluate the biological and toxicological as side effects (direct and indirect effects) of this compound on the predatory mite, *P.finitimus* under laboratory conditions.

MATERIALS AND METHODS

Tested compound:

Pyriproxyfen (Admiral 10% EC), is a Juvenile hormone Mimic (JHM)., 4-phyenoxyphenyl (RS) -2- (2-Pyridyloxy) prapyl ether, produced by Sumitomo Chemical Co. Japan.

Rearing of *Tetranychus urticae*:

A colony of the two spotted spider mite TSSM, *T. urticae* was collected from infested leaves of cotton plants from Qaliubia governorate and reared in the laboratory of Plant Protection Research Institute, under constant conditions of 25 ± 2° and 65 ± 5 % RH. A pure culture of TSSM was propagated on sweet potato cuttings placed in glass jars filled with water, which in turn were placed in water pan and protected by an arch covered with muslin, the adult female of TSSM used as a prey.

Rearing of the predatory mite, *Phytoseius finitimus:*

The predatory mite, *P. finitimus* was obtained from infested cotton plants in Qualiubia governorate; it was transferred to the laboratory and reared on TSSM adults maintained on sweet potato cuttings. Adult females were allowed to lay eggs and the obtained eggs were used to establish main culture of these mites.

Toxicological studies:

To examine the direct and indirect effects of pyriproxyfen against adult females and protonymphs of the predatory mite, *P. finitimus*. Three aqueous concentrations 2.5, 5 and 10 ppm were used by diluting the formulated compound with distilled water. Six cotton leaf discs were considered as three replicates for each concentration. Sixty newly emerged adult females or protonymphs were used for each replicate. Leaf discs 3 cm were placed upside down on moisten cotton wool in Petri dishes 10 cm. Leaf discs surface carrying the different stages of the mite were directly sprayed using hand glass atomizer. Other three replicates were sprayed with distilled water as control. All treatments were kept under laboratory conditions of 25 ± 2°C and 65 ± 2 RH %. Dead stages were counted, with the aid of a dissecting stereomicroscope, after 24, 48 and 72 hrs. The average mortality % was calculated and corrected using Abbott's formula (1925). The LC_{50}, LC_{90} and slope values were statistically calculated according to Finney (1971) using computer software program.

Biological studies:

The treated females of the predatory mite, *P. finitimus* were examined daily to determine different biological aspects such as: developmental duration periods, oviposition periods, female fecundity and egg viability. Males and females longevity, the life –cycle and the life-span were also determined.

RESULTS AND DISCUSSION

Direct effect of pyriproxyfen 10% EC against different developmental stages of the predatory mite , *P. finitimus:*

Toxicity response of protonymphs and adult females of the predatory mite, *P. finitimus* treated with three concentrations of pyriproxyfen 10% EC (2.5 ,5 ,10 ppm) are shown in Table (1) .

Data Table (1) indicated that pyriproxyfen showed no direct harmful effect on both protonymphs and females of the predatory mite, *P. finitimus* according to the mortality percentages recorded for LC_{50} and LC_{90} of the tested compound. The maximum concentration (10 ppm) showed low toxicity, it caused 12.77% and 10.56% on protonymphs and females, respectively after 72 hr post exposure, so pyriproxyfen considered as a non toxic agent for the

tested predatory mite, *P. finitimus* according to quantitative toxicity categories determined by the International organization for Biological control for toxicity assessment to predatory and phytophagous mites: non toxic (< 25 mortality%), slightly toxic (25-50%), moderately toxic value(51-75%) and highly toxic (>75%) (Hassan*.,* 1985). The LC_{50} values were 80.24 ppm and 217.34 ppm on protonymphs and females, respectively. Also, LC_{90} values were 698.5 ppm and 3501.14 ppm against protonymphs and females, respectively.

Table (1): Mortality percentages , LC50, LC90 and slope of *P. finitimus* protonymphs and females treated with three concentrations of pyriproxyfen 10 %EC.

Conc. (ppm)	mortality % after treatment					
	24 hr.		48 hr.		72 hr.	
	Proto nymph	females	Proto nymph	females	Proto nymph	females
2.5	1.1	2.22	3.3	3.3	4.44	5.0
5	4.44	3.33	5.6	5.6	10.0	7.78
10	3.89	3.89	7.22	7.22	12.78	10.56
Control	0.0	0.0	1.67	0.0	3.33	0.0
	LC after 72 hr. (ppm)					
	LC50 ppm		LC90 ppm		Slope	
	80.24	217.34	698.5	3501.14	1.364	1.662

In a previous study (Abdel- Hafez, *et al.,* (2014) evaluated the same three concentrations i.e., 2.5, 5 ,10 ppm of pyriproxyfen 10 % EC against the two spotted spider mite, *T .urticae* under laboratory conditions , an d found that the tested compound exhibited high direct contact toxicity on protonymphs and females of *T.urticae*, according to the values of LC_{50} and LC_{90} , where the LC_{50}'s were 4.21ppm and 4.36 ppm after 72 hr of treating for protonymphs and females, respectively, in addition the LC_{90} values were 11.59 ppm and 13.04 ppm for protonymphs and females, respectively (Abdel –Hafez, *et al* 2014).

These results are in agreement with those obtained by Rani and Mohan (1998) who found that the insect growth regulator, flufenoxuron (Cascade 10% DC) at all tested dosages (0.5, 1.0, 1.5 and 2.0 ml/litre) significantly controlled *T.urticae* stages on roses under screen house conditions in Karnataka, India. The most effective dosage was 1.5 ml / liter, which produced more than 90% mortality and it was non toxic to rose plants and safe to the predatory mite, *Amblyseius* spp. Also, flufenoxuron was much less toxic to adult females and immatures of the predatory mite, *Phytoseiulus persimilis* than to those of *Tetranychus urticae* (Sangsoo and Sangsun, 2002).

However, Halloum and Qerhaili (2013) indicated that, diflubenzuron was slightly toxic to the two phytoseiidae predatory mites, *Neoseiulus fallacies* and *Typhlodromus cotoneastri* under laboratory conditions.

Indirect effects of JHM, pyriproxyfen against different developmental stages of the predatory mite, *P. finitimus:*

Effect of the candidate compound on the duration periods of the developmental stages of the predatory mite, *P. finitimus* in 1^{th} and 2^{nd} offspring are presented in Tables (2 and 3).

Incubation period:

The incubation period of *P. finitimus* eggs, which resulted from female treated with three concentrations of pyriproxyfen showed slightly decrease, except the concentration of 2.5 ppm, it produced the same incubation period of females and males, recording 2.15 days for females and 1.71 days for males in the 1st offspring compared with 2.15 days for females and 1.71 days for males of the control. Also the concentration 2.5 ppm enhanced the same incubation period for females and males in the 2nd offspring.

Larval period:

As shown in Tables (2 and 3) all tested concentrations of pyriproxyfen revealed slightly affect on the larval period of the predatory mite. The highest concentration (10 ppm) caused slightly increase in larval period of both males and females, it increased from 1.89 days for females in untreated female to 1.95 days for females and from 1.44 days for males in control to 1.59 days for males. However, the tested compound didn't affect on the larval period of the predatory mite in the 2nd offspring.

Protonymphal period:

The obtained results in Tables (2 and 3) showed slightly effect on the protonymphal period of males and females of the predatory mite. The concentration 2.5 ppm gave slightly increase in protonymphal period of females, it was 2.59 days for females compared with 2.52 days for females in control. Also the highest concentration 10 ppm gave 2.18 day for males compared with 2.22 days in control. Also pyriproxyfen slightly affected on protonymphal period of the predatory mite at all tested concentrations in the 2nd offspring as shown in Tables (2and 3).

Deutonymphal period:

All tested concentrations of pyriproxyfen slightly affected on the deutonymphal of females and males of the predatory mite in the 1th and 2ndoffspring Tables (2 and 3).

Life cycle:

The mean life-cycle duration of female and male immature stages resulted from treated females with three concentrations of pyriproxyfen in 1th and the 2nd offspring are recorded in Tables (2 and 3). All tested concentrations slightly affected the life cycle of males and females. The maximum concentration , 10 ppm caused slightly increase in the life cycle of females, it recorded 9.91 days compared with 9.57 days in untreated females, while it hadn't any effect on life-cycle of males in the 1th offspring (Table 2). However the concentration 5 ppm gave slightly increase in life cycle of females it recorded 9.99 days compared with 9.56 days in control, while the concentration , 10 ppm caused slightly increase in life cycle of males, it gave 7.8 days in treated males compared with 7.51 days in control in the 2nd offspring Table (3).

Table (2): Efficiency of pyriproxyfen 10% EC on duration periods of life cycle of the predatory mite, *P. finitimus* in the 1th offspring.

Sex	Stage	Duration (days) mean ±S.E			Control
		2.5 ppm	5 ppm	10 ppm	
Female	Incubation period	2.15± 0.0078	2.12± 0.0047	2.13 ± 0.007	2.15 ± 0.011
	Larva	1.87 ± 0.045	1.77 ± 0.014	1.95 ± 0.009	1.89 ± 0.019
	Protonymph	2.59 ± 0.028	2.54 ±0.039	2.47± 0.0115	2.52 ±0.009
	Deutonymph	3.13 ± 0.012	3.1 ± 0.022	3.36 ± 0.126	3.14 ± 0.012
	Life cycle	9.74 ± 0.051	9.53 ± 0.068	9.91 ±0.134	9.57 ± 0.126
Male	Incubation period	1.71± 0.05	1.6 ± 0.0276	1.87±0.0226	1.71 ± 0.106

	Larva	1.59 ± 0.103	1.5 ± 0.023	1.38± 0.0268	1.44 ± 0.022
	Protonymph	2.15 ± 0.009	2.18 ±0.0076	2.18±0.011	2.22 ±0.0125
	Deutonymph	2.36 ± 0.016	2.49± 0.0245	2.54 ± 0.01	2.45 ± 0.026
	Life cycle	7.61 ± 0.046	7.82 ± 0.052	7.88 ±0.166	7.8 ±0.097

Table (3): Efficiency of pyriproxyfen 10% EC on duration periods of life cycle of the predatory mite, *P. finitimus* in the 2nd offspring.

Sex	Stage	Duration (days) mean±S.E			Control
		2.5 ppm	5 ppm	10 ppm	
Female	Incubation period	2.12± 0.0064	2.25± 0.1	2.13± 0.0078	2.12± 0.0098
	Larva	1.81 ± 0.023	1.9 ± 0.024	1.8± 0.035	1.8 ± 0.0
	Protonymph	2.63 ± 0.01	2.51 ±0.05	2.64± 0.0178	2.53 ±0.054
	Deutonymph	3.12± 0.0079	3.34 ±0.121	3.12± 0.0051	3.1 ±0.0027
	Life cycle	9.67±0.025	9.99± 0.101	9.61± 0.131	9.56 ±0.048
Male	Incubation period	1.61±0.022	1.44±0.028	1.83±0.025	1.6±6.094
	Larva	1.36 ±0.013	1.42 ±0.0061	1.42± 0.0065	1.4 ±0.018
	Protonymph	2.13 ± 0.01	2.14 ±0.009	2.13±0.013	2.2 ±0.080
	Deutonymph	2.29±0.016	2.44±0.02	2.43±0.0075	2.41 ±0.056
	Life cycle	7.23±0.181	7.45±0.035	7.8±0.027	7.51±0.0145

Oviposition periods and fecundity:

Oviposition periods and female fecundity of *P. finitimus* treated with three tested concentrations of pyriproxyfen in the 1th and 2nd generation are presented in Tables (4 and 5).

Pre-oviposition period:

Results indicated that the time required for maturation of females mite ovaries treated with pyriproxyfen exhibited no indirect harmful effects on pre-oviposition period compared with untreated females in both 1th and 2nd offspring (Tables 4 and 5).

Oviposition period:

Also, the mean oviposition period exhibited no harmful effects when adult females of the predatory mite, *P. finitimus* which were treated with all tested concentrations of pyriproxyfen as shown in Tables (4 and 5).

Post-oviposition period:

Also pyriproxyfen showed no indirect harmful effects on adult female post – oviposition period of the predatory mite, *P. finitimus* in both 1th and 2nd offspring as shown in Tables (4 and 5).

Female fecundity:

The tested compound exhibited no indirect effects on the number of laid eggs/ ♀ of the predatory mite*; P. finitimus*, it gave the same number of egg/♀ in untreated females approximately in both 1th and 2nd generation as shown in Tables (4 and 5). Also the tested compound exhibited no harmful effects showed on the viability of eggs deposited by the treated females compared with untreated females in 1th and 2nd offspring (Tables 4 and 5).

Adult longevity:

The tested compound exhibited no harmful indirect effects on males and females longevity resulted from treated females with pyriproxyfen in 1th and 2nd generation as shown in Tables (6 and 7).

Life span:

Results clearly showed no harmful effects on the life span of both males and females of the predatory mite, *P. finitimus* treated with the tested compound (Tables 6 and 7).

Table (4): Effect of pyriproxyfen 10% EC on oviposition periods and fecundity of *P. finitimus* female in the Ith offspring.

Conc. ppm	Oviposition period mean ± S.E (days)			Fecundity mean± S.E	
	Pre-oviposition	Oviposition	Post-oviposition	Egg/ female	% Hatchability
2.5	2.63 ± 0.0198	18.24 ± 0.171	3.1 ± 0.071	41.83± 0.366	95.22%
5	2.67 ± 0.0379	18.13± 0.0278	2.98 ± 0.04	41.5 ± 0.391	95.18%
10	2.66 ± 0.023	18.26 ± 0.170	3.38 ± 0.107	41.17± 0.366	92.86%
Control	2.65 ± 0.026	18.3 ± 0.122	3.38 ± 0.172	42.67± 0.272	97.62%

Table (5): Effect of pyriproxyfen 10% EC on oviposition periods and fecundity of *P. finitimus* female in the 2nd offspring.

Conc. ppm	Oviposition period mean ± S.E (days)			Fecundity mean± S.E	
	Pre-oviposition	Oviposition	Post-oviposition	Egg/ female	% Hatchability
2.5	2.43 ± 0.023	18.0 ± 0.11	3.17 ± 0.12	41.17 ± 0.436	88.67%
5	2.39 ± 0.0093	18.23± 0.106	3.21 ± 0.03	41.3 ± 0.385	87.15%
10	2.57 ± 0.033	18.23 ± 0.125	3.3 ± 0.107	41.5 ± 0.391	83.15%
Control	2.53 ± 0.027	18.27 ± 0.072	3.05 ± 0.147	41.67 ± 0.272	89.96%

Table (6): Effect of pyriproxyfen 10% EC on adult longevity and life span of *P. finitimus* males and females in 1th offspring.

Conc. Ppm	Longevity Mean ± S.E (days)		Life span Mean ±S.E (days)	
	female	male	female	male
10	24.19±0.176	19.53±0.0096	33.96 ±0.134	27.37 ±0.095
5	23.78 ±0.066	19.09 ±0.038	33.31± 0.089	26.89± 0.052
2.5	23.98± 0.204	19.34±0.082	33.71 ±0.216	26.93 ±0.082
Control	24.31± 0.177	19.5 ± 0.024	33.88± 0.139	27.3 ±0.121

Table (7): Effect of pyriproxyfen 10% EC on adult longevity and life span of *P. finitimus* males and females in 2th offspring.

Conc. ppm	Longevity Mean ± S.E (days)		Life span Mean ±S.E (days)	
	female	male	female	male
10	24.03±0.159	19.66±0.018	33.65 ±0.163	27.48±0.027
5	23.21 ±0.119	19.08 ±0.046	33.21± 0.166	26.53± 0.054
2.5	23.5 ±0.144	18.97 ±0.09	33.16 ±0.129	26.19 ±0.184
Control	23.85 ± 0.147	18.38 ±0.513	33.41 ±0.187	26.73±0.223

In a previous study of Abdel- Hafez, *et al.* (2014)) who tested the toxicity of this on *T. urticae* stages under laboratory conditions , and found that pyriproxyfen had contact toxicity against different developmental stages of this pest which stop feeding within few hours of treatment and die 2-3 days post exposure. No larval, and nymphal molting was observed. In addition it had latent effects on female oviposition and fecundity, i.e., decreased the number of eggs laid/♀ and increased both adult ♂ and ♀ longevity (Abdel-Hafez, *et al.* 2014) . The present investigation was carried out to study the side effects of this compound against the predatory mite, *P. finitimus*. Data clearly revealed that pyriproxifen exhibited no direct effects (morality) on both protonymphes and females of the mite according to mortalities and LC_{50} values. In addition, it showed no indirect harmful effects on developmental time, oviposition period /female, male and female longevity, fertility and eggs viability of the mite under laboratory conditions. It could be concluded that pyriproxifen caused high toxicity on the TSSM ,*T. urticae* and safe on its predatory mite , *P .finitimus* . Our results agree with previous studies of those mentioned by El-Banhawy & Amer (1992), Rani & Mohan (1998) and Sangsoo &Sangsun (2002), who indicated that the insect growth regulator, flufenoxuron was much less toxic to the adult females and immature of the predatory mite, *Phytoseiulus persimilis* than to those of TSSM, *T. urticae.* Female predators produced 84-96% as many egg as did of control females, fecundity, prey consumption and the sex ratio of the progeny were not substantially affected. They suggested that, flufenoxuron appeared to be promising candidates for use in Integrated mite Management Programs, where, *P.persimilis* is the major natural enemy. On the other hand, pyriproxyfen was demonstrated to has no indirect harmful effects on adult female oviposition and egg viability of the green lacewing *Chrysoperla carnea.* (Medina *et al.,* 2003 and Suma, *et al.,* 2009).

Similarly pyriproxyfen exhibited no indirect harmful effects on development time, female longevity, and fertility of *Orius spp* after exposure under laboratory conditions. Pyriproxyfen didn't indirectly impact *Delphastus catalinae* female fecundity after adults had fed upon treated eggs of the sweet potato. Whitefly, *Bemisia tabaci* (Liu and Stansly, 2004). Pyriproxyfen, had no indirect effects on the predatory bugs, *Orius spp.* with no harmful effects on adult female oviposition and egg viability (Nagai, 1990). Pyriproxfen is also non-toxic to the predatory bugs, *Orius Laevigatus* via ingestion and residual contact (Delbeke, *et al.,* 1997).

These results suggest that pyriproxyfen compound could be a good alternative and interesting incorporation in Integrated program Strategy for controlling TSSM, *T. urticae* due to high toxicity on target pest and has no indirect harmful effects on its natural enemies to help minimizing the usage of conventional chemical pesticides.

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التأثيرات الجانبية لمشابه هرمون الحداثة، البيروبروكسفين على المفترس الأكاروسى *Phytoseius finitimus* (Acari: Phytoseiidae)

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الملخص العربى

تم دراسة التأثيرات الجانبية المباشرة والغير مباشرة لمشابه هرمون النمو البيروبروكسفين على المفترس الأكاروسي *Phytoseius finitimus* تحت الظروف المعملية حيث تم تقييم التأثيرات المباشرة للمركب المختبر كنسب الموت بثلاثة تركيزات مختلفة (٥و٢، ٥ ، ١٠ جزء فى المليون)على الأطوار الكاملة والغير كاملة من المفترس الأكاروسي ، كما تم تقييم التأثيرات المتأخرة للمركب على بعض المظاهر البيولوجية لدورة الحياة للأطوار الكاملة والكفاءة التناسلية وفترة ما قبل وضع البيض وما بعد وضع البيض وفترة وضع البيض وحيوية البيض ودورة الحياة ، بالاضافة الى فترات الأطوار الغير كاملة للإناث والذكور.

أوضحت النتائج أن المركب المختبر أعطي تأثيرات منخفضة السمية على الأطوار المختلفة المعاملة من المفترس الأكاروسي حيث أعطي أعلى تركيز مختبر وهو ١٠ جزء في المليون ١٢.٧٧٪ موت على طور الحورية الأولي ، بينما أعطي ١٠.٥٦٪ موت على الأفراد الكاملة بعد ٧٢ ساعة من المعاملة ومن جهة أخرى ليس للمركب المختبر أي تأثيرات جانبية متأخرة على دورة الحياة وفترات وضع البيض وطول عمر كلا من الإناث والذكور ، كما اثبتت النتائج المتحصل عليها عدم وجود تأثير على الكفاءة التناسلية وحيوية البيض لجميع التركيزات المختبرة.

تقترح الدراسة الحالية إستخدام مشابه هرمون الحداثة (البيروبروكسفين) ضمن برامج المكافحة المتكاملة للعنكبوت الأحمر ذو البقعتين *Tetranychus. urticae* وذلك لما له من تأثير عالي على الآفة المستهدفة ودرجة أمان عالية على المفترس الأكاروسى المصاحب للأفة كما ان استخدام مثل هذه المواد تساعد في ترشيد استهلاك المبيدات الكيميائية التقليدية.