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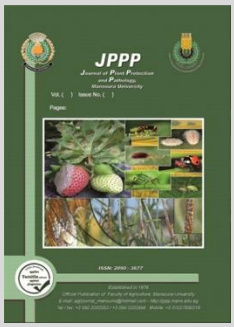
Insecticidal and Repellent Activity of some Pesticides and Plant Oils against Alfaalfa Weevil, *Hypra brunneipennis* (Boheman) (Coleoptera: Curculionidea).

Ali, A. M.*

Plant Protection Department, Faculty of Agriculture, Minia University, Minia, Egypt.



ABSTRACT

The Alfaalfa weevil, *Hypera brunneipennis* is a major pest of alfalfa in Egypt. In this research two pesticticides (Emamectin benzoate α Neeemix) and two plant oils (Camphor α Coriander) were evaluated for their efficiency in the control of the secand larval instar and adults of *H. brunneipennis* under laboratory condition.The LC_{50} and LC_{90} values of these compounds against the 2nd instar larvae and adults of *Hypera brunneipennis* were calculated at 24, 48, 72 and 96 hours.According to the LC_{50} and LC_{90}, it could be concluded that the highest potent compound was Emamectin benzoate followed by Neemix. On the other hand, in choice tests with adults of this pest (LC_{25} concentration) the highest repellency properties was with Neemix and the plant oil of Camphor treatment (5 and 17.5% weevils in treated side, respectively), followed by Coriander plant oil (25%), But, the treatment with Emamectin benzoate have significantly less repelling properties (55.8%) after 72 hr post treatment.

Keywords: *Hypera brunneipennis*, Insecticidal activity, Repellent activity, Plant oils, Emamectin benzoate, Neeemix.

INTRODUCTION

Alfalfa is most valued and grown forage world wide plant due to its being wide adaptation, biological nitrogen fixation, soil improvement and benefits to subsequent crops. Additionally, the plant provides high energy and safe feed in a high demand by meat and daily industry of wide range of ruminant live stock (such as cows, sheep and goats).

However, the Egyptian alfalfa weevil (EAW), *Hypera brunneipennis* (Boheman) (Coleoptera, Curculionidae) is considered to be the most serious and destructive pest of alfalfa in Egypt (Al-Doghairi and El Hag, 2003). In the same time, the larvae and adults of alfalfa weevil are voracious feeders damaging foliage and a new grown shoot of alfalfa plant (Neima and Mustafa, 2016). In fields with severe infestation may appear silver on white, because most alfalfa leaves consumed, retarding new growth and resulting in low plant vigor and yield in subsequent harvest (Ignoffo CM,1975).

The objective of this study to investigate the insecticidal and repellent activity of two biopesticides (Emamectin benzoate α Neeemix) and the two plant oils (Camphor α Coriander) to substitute synthetic chemical pesticides with these treatment methods as a part of integrated pest management and avoid using synthetic insecticides and reduce its risks to people, water resource and wild life.

MATERIALS AND METHODS

Test insects: Alfalfa weevil larvae were collected early in the morning, with a sweep- net from alfalfa field at the college of agriculture Minia Univ., Egypt.

Larvae were stored out in the laboratory, maintained on fresh alfalfa plants kept in water and the second larval instar were selected later for the tests (Al-Doghairi and El Hag, 2003). Also, the alfalfa weevil adults were collected from the same previous field and they were taken to the laboratory for testing.

Pesticides and plant oils tested: Two pesticides (Emamectin benzoate α Neeemix) and the two plant oils (Camphor α Coriander) were applied. The rates tested were 200,400,600,800 and 1000pm for the Pesticides and plant oils. Then, 0.2% of Tween 80 added to each plant oil surfactant for each concentration and to the water control as well.

Toxicity tests: Fresh alfalfa stems were collected and brought into the laboratory where intact leaflets were detached. Leaflets were then dipped into the desired concentration, they dried in the laboratory and each was placed in a Petri – dish. Ten EAW larvae were introduced into each Petri – dish which represented one replicate. The leaflets in controls were dipped with water with surfactant. Each treatment was replicated four times in a completely randomized design. Dishes were kept in the laboratory during the bioassay period under 25 ± 2 Co , RH 60-70%. The same procedure was followed by using the adults of this pest. The mortality was counted after 24, 48, 72 and 96 hours later for each test (Khaphy A.A, 1981, DePew LP and SloderBeck PE 1985).

Repellent test: Repellence was evaluated when adults of Hypera brunneipennis were given the opportunity to choose between treated and untreated leaves using the technique described by (ibrahim et al. 2004) with little modifications. Twelve chambered transparent plastic containers (27.6 by 18.2 by 3.8 cm each box, 6.1 by 3.8 cm each chamber) were used. The four subunits having two lateral side chambers were chosen as replications for each treatment. Small holes (0.5 cm diameter) were made of bottom of each of the two inner walls connecting the three chambers of each subunit to

* **Corresponding author.**
E-mail address: ali30785@yahoo.com
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allow adults to freely move between the three chambers. For each tested chemical sublethal LC25 was descriminated.The concentration was prepared by dessolving the (128.82, 181.97, 234.42 and 251.19ppm) from (Emamactin benzoate, Neemix, Camphor oil and Coriander oil respectively) in 500 ml water.The four subunits were used for each concentration as replications in wich in the side marked "treated" Five treated leaves from Alfalfa (after dryness as previosely described) were placed.However, Five leaves from fresh alfalafa were placed in the side market untreatment (Control) after dipping in 500 ml water.Ten adults were released in the meddle chamber of each subunit.The containers were covered with thier lids and an opaque dark blue sheet to prevent the possible effect of light on insect oriantation.Number of adults were counted in the treated,meddle and untreated chambers after 24,48 and 72 hour. Compareson between mean number of adults in treated and untreated side by using paired T-test was observed.

Data analysis: In the first test, the data of the second larval instar and adults stage mortality were corrected by the Abbot formula (Abbott, 1925) and analyzed by probit analysis using the software SPSS 22 and LC50 and LC90 values were calculated. Also, the toxicity index of the tested compounds was determined according to Sun (1950) as follow: Toxicity index = (LC50 or 90 of the most effective compound/ LC50 or 90 of the tested compound) *100

RESULTS AND DISCUSSION

A: Toxicity tests:

Data in Table (1) and Fig (1) showed that the results of probit analysis of the different concentrations for the two pesticides (Emamectin benzoate α Neemix) and the two plant oils (Camphor α coriander) against the second larval instar of the alfalfa weevil, *H. brunneipennis* after 24,48,72 and 96 hr post treatment. From these results, it cleared that increasing the time of exposure, decreased the values of LC_{50} and LC_{90} for all treatments.

Table 1. Probiotic analysis of different concentrations of two pesticides (emamectin benzoate α neemix) and two plant oils (camphor α coriander) on the second larval instar of *Hypra brunneipennis* in the laboratory after different times.

Treatment	Time(h)	Line Equation	Slope ± SE	LC_{50} (ppm)	LC_{90} (ppm)	TI-(LC_{50})	TI-(LC_{90})
Emamectin benzoate	24	y = 3.0968x - 3.4612	3.0968 ± 0.89	537.03	1412.54	100	83.18
	48	y = 2.4048x - 1.5076	2.4048 ± 0.99	512.86	1698.24	95.49	77.63
	72	y = 2.9524x - 2.5288	2.9524 ±1.03	354.81	954.99	100	100
	96	y = 3.05x - 2.115	3.05 ±1.37	213.79	562.34	100	100
Neemix	24	y = 2.9468x - 3.2712	2.946 ± 0.94	645.65	1737.80	83.18	67.61
	48	y = 2.9909x - 3.0573	2.9909 ± 0.87	489.78	1318.26	100	100
	72	y = 2.969x - 2.8105	2.969 ± 0.98	426.57	1148.15	83.18	83.18
	96	y = 2.8632x - 2.1421	2.8632 ±1.33	309.03	724.44	69.18	77.62
Camphor oil	24	y = 4.8857x - 9.0471	4.8857 ±1.64	758.57	1380.38	70.79	85.11
	48	y = 2.9162x - 2.9842	2.9162 ± 0.88	549.54	1513.56	89.13	87.09
	72	y = 3.1805x - 3.537	3.1805 ± 0.88	478.63	1230.27	74.13	77.62
	96	y = 3.3381x - 3.591	3.3381 ±1.04	371.54	912.01	57.52	61.66
Coriander oil	24	y = 7.75x - 17.515	7.75 ± 2.80	812.83	1174.89	66.07	100
	48	y = 2.5805x - 2.327	2.5805 ± 0.90	691.83	2187.76	70.79	60.26
	72	y = 2.5344x - 2.0896	2.5344 ± 0.87	616.59	1995.26	57.54	47.86
	96	y = 2.9571x - 2.7714	2.9571 ±1.01	426.58	1148.15	50.12	48.98

Toxicity index (TI) of the tested compounds was determined according to Sun (1950) as follows: Toxicity index = (LC_{50} or $_{90}$ of the most effective compound/ LC_{50} or $_{90}$ of the tested compound) *100

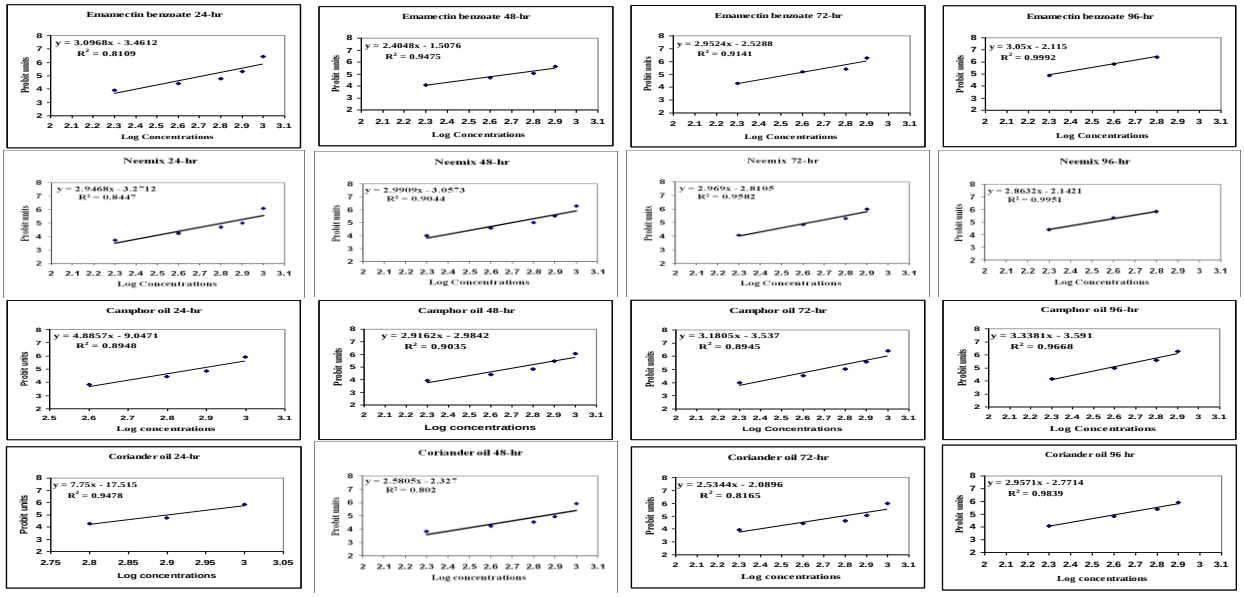

Fig 1. Toxicity lines established from plotting the probit units corresponding to 24, 48,72 and 96h mortality percentages of the second larval instar of *Hypra brunneipennis* in the laboratory versus concentration logarithms of tested chemicals.

Moreover, the lowest LC_{50} and LC_{90} values (213.79 α 562.34ppm) was calculated for Emamectin benzoate, followed by the other tested compounds of Neemix,Camphor and Coriander, However, the values of LC_{50} and LC_{90} (309.03 α 724.44 ppm), (371.54 α 912.01 ppm) and (426.58 α 1148.15 ppm),respectively after 96 hr of exposure.

According to the calculated toxicity index (TI), it observed that Emamectin benzoate was the highly effective compound (100% TI after 24, 72 and 96 hr post treatment for LC_{50} and after 72 and 96 hr for LC_{90} followed by Neemix, which gave 100% TI for LC_{50} and LC_{90} after 48 hr post treatment. In similar research, Neima and Mustafa, 2016 examined three types of plant extracts against the Alfalfa weevil *Hypera postica* under laboratory condition and they showed that Neem leaves extract was the most effective, causing 73.33% larvae mortality after 4 days and 96.66% adult mortality within 8 days after treatment. Moreover, Tang and Weathersbee, 2002 and Reddy ,2016 showed a good relationship between mortality percentage of *H. postica* and number of days after treatment.

In the same time, (Fig, 2) cleared that among all the tested compounds, Emamectin benzoate and Neemix caused 100% mortality for *H. brunneipennis* larvae after 72 and 96 hr post treatment at 800 and 1000 ppm concentration. While, the other tested compounds of Camphor and coriander oils took 96 hr to kill 100% mortality of *H. brunneipennis* larvae at only the concentration of 1000 ppm. (Haider *et al.*, 2017).

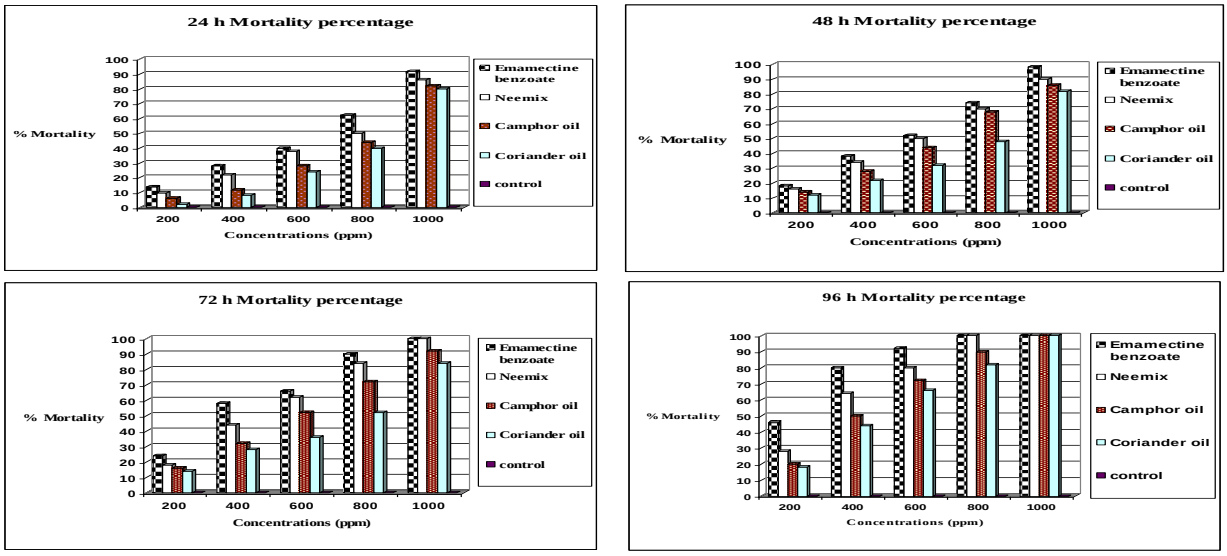

Fig 2. Mortality Percentage of the second larval instar and time changes with different concentrations for all treatments.

Data in Table (2) and Fig (3) cleared the probit analysis of different concentrations for all tested compounds concerning the adult stage of alfalfa weevil, However after 96 hr post treatment all the compounds gave the lowest LC_{50} and LC_{90}. Moreover, Emamectine benzoate gave the lowest value of LC_{50} and LC_{90} (223.87α 616.59ppm), and Neemix (338.84 α 870.96 ppm), followed by plant oils of Camphor (426.58 α 1047.13ppm) and Coriander oil (478.63 α 1288.25 ppm), respectively.

Table 2. Probiotic analysis of different concentrations of two pesticides (emamectin benzoate α neemix) and two plant oils (camphor α coriander) on the adult of *Hypra brunneipennis* in the laboratory after different times.

Treatment	Time(h)	Line Equation	Slope ± SE	LC_{50} (ppm)	LC_{90} (ppm)	TI-(LC_{50})	TI-(LC_{90})
Emamectin benzoate	24	y = 2.8452x - 3.1177	2.8452 ± 0.97	724.44	1995.26	100	61.66
	48	y = 2.8811x - 2.9335	2.8811 ± 0.89	562.34	1548.82	100	97.92
	72	y = 2.6891x - 1.9943	2.6891 ± 0.98	398.11	1202.26	100	100
	96	y = 2.8646x - 1.7402	2.8646 ± 1.32	223.87	616.59	100	100
Neemix	24	y = 4.6629x - 8.409	4.6629 ± 0.86	758.58	1412.54	95.49	87.09
	48	y = 2.9064x - 3.096	2.9064 ± 0.91	616.59	1698.24	91.20	89.19
	72	y = 2.9735x - 2.9697	2.9735 ± 1.04	478.63	1288.25	83.18	93.33
	96	y = 3.1021x - 2.8525	3.1021 ± 1.02	338.84	870.96	66.07	70.79
Camphor oil	24	y = 4.8192x - 9.0061	4.8192 ± 1.70	812.83	1479.11	89.13	83.18
	48	y = 2.8824x - 3.093	2.8824 ± 0.93	645.65	1778.28	87.09	85.11
	72	y = 3.0587x - 3.3817	3.0587 ± 0.91	549.54	1445.44	72.44	83.18
	96	y = 3.2684x - 3.5814	3.2684 ± 0.89	426.58	1047.13	52.48	58.88
Coriander oil	24	y = 7.8865x - 18.093	7.8865 ± 2.88	851.14	1230.27	85.11	100
	48	y = 4.1794x - 7.0121	4.1794 ± 1.55	741.31	1513.56	75.86	100
	72	y = 2.5402x - 2.1771	2.5402 ± 0.88	676.08	2137.96	58.89	56.23
	96	y = 2.961x - 2.9237	2.961 ± 1.04	478.63	1288.25	46.77	47.86

Toxicity index (TI) of the tested compounds was determined according to Sun (1950) as follow: Toxicity index = (LC_{50} or $_{90}$ of the most effective compound/ LC_{50} or $_{90}$ of the tested compound) *100

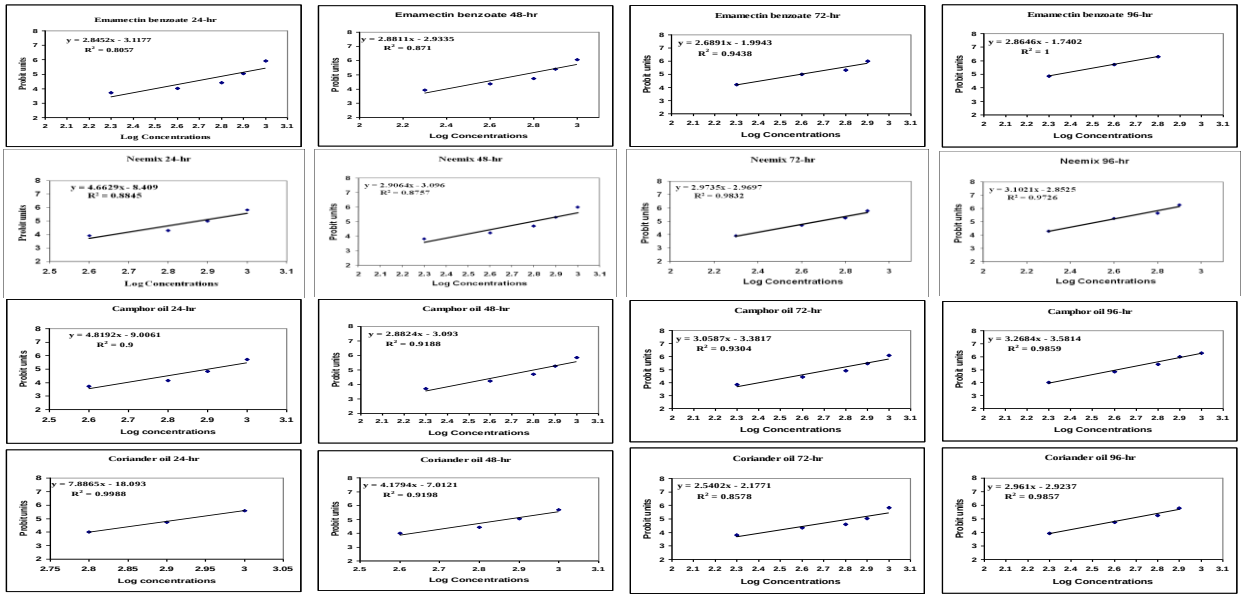

Fig 3. Toxicity lines established from plotting the probit units corresponding to 24,48,72 and 96h mortality percentages of adults of *Hypra brunneipennis* in the laboratory versus concentration logarithms of tested chemicals.

According to the calculated Toxicity index (TI), it observed that Emamectin benzoate was the highly effective compound (100% TI after 24, 48,72 and 96 hr of exposure time for LC_{50}), but the TI for LC_{90} the same compound gave 100% TI only after 72 and 96 hr post treatment.(Table, 2).

On the other hand, among all the tested compounds, Emamectin benzoate and Neemix caused 100% mortality of *H. brunneipennis* adults at 72 and 96 hr post treatment at 800 and 1000ppm (Fig. 4).In comparison, the other tested compounds such as Camphor and Coriander oils caused 100% TI after 96 hr post treatment only at the concentration of 1000 ppm.

In general, it could be concluded that the adults of *H. brunneipennis* was highly tolerant than larvae of this pest at all tested compounds when compared the LC_{50} and LC_{90} at all times of exposure. (Tables 1 and 2).

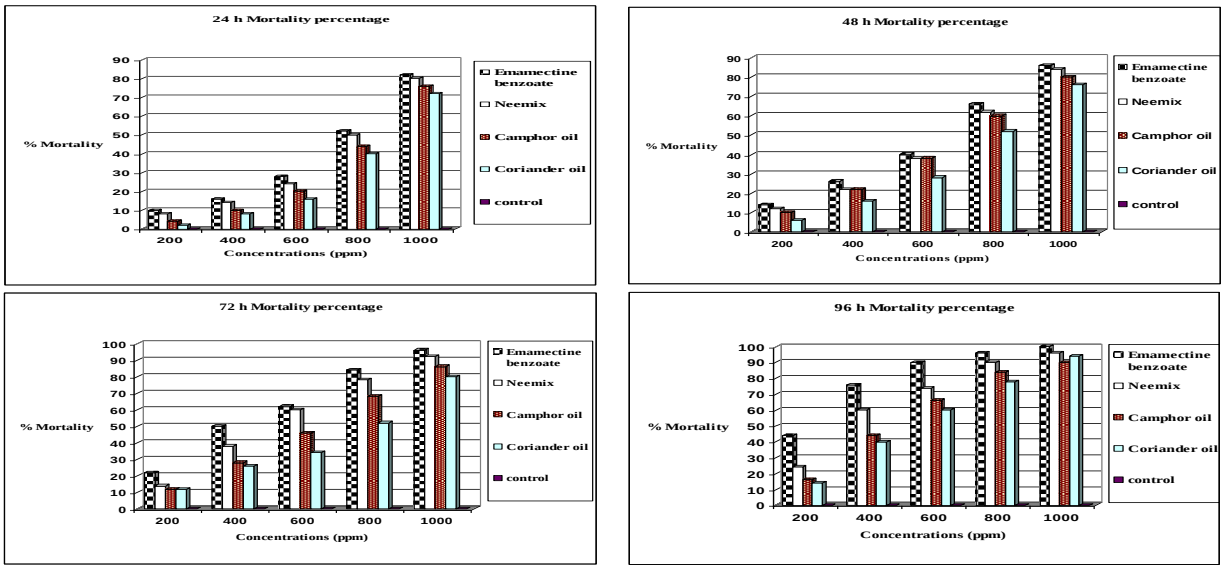

Fig 4. The adult mortality Percentage and time changes with different concentrations for all treatments.B: Repellency

In the current study, newly emerged adults were given the opportunity for 3 days to choose between treated and untreated leaves of alfalfa. One concentration was selected (LC_{25}) to conduct this study. Data in Table (3) and Fig (5). Showed that, the greatest repellency was with Neemix followed by Camphor and Coriander oils (0.5. 1.7 and 2.5 weevils in the treated leaves side, respectively). In contrast, the greater numbers of insects were in the lateral side chambers having untreated leaves that were significantly greater than those in the corresponding sides of the control. However, more than 60% of weevils oriented to the untreated sides of Neemix, Camphor and Coriander oil that were significantly greater those in the corresponding sides of control. In contrast, the treatment with Emamectin benzoate, the differences between the number of alfalfa weevil adults in the treated leaves side were insignificantly with those in the corresponding side of the control (Table.3 and Fig.5). In similar results, Ahmed

& Grainge, 1986; Schmutterer, 1990 and Mordue & Nisbet, 2000, showed that, Azadirachtin, (a chemical complex found in seeds of neem, *Azadirachta indica* A. Juss), Also, has repellent effect. *Cinnamomum camphora*, Lauraceae (is a Chinese medicinal plant widespread in China) . The essential oils, compounds and their antimicrobial, antifungal insecticidal as well as repellent activity has been reported (Liu *et al.,* 2001; Liu *et al.*, 2006 ; Wang *et al.,* 2005 and Mann *et.,al*. 2010)

Table 3. Mean number (± SD) of *Hypra brunneipennis* adults (± SD) when given the opportunity to choose between two treated side chambers having un-treated and treated alfafa leaves with the tested compounds at concentration corresponding to the 96-hr. LC_{25}.

Treatments	24 hr				48hr				72 hr			
	Treated side	Meddle side	Untreated side	paired *t*-test (P-value 0.05)	Treated side	Meddle side	Untreated side	paired *t*-test (P-value 0.05)	Treated side	Meddle side	Untreated side	paired *t*-test (P-value 0.05)
Control	4.17 ± 1.19	2.08 ± 1.31	3.75 ± 1.36	0.658 NS	4.67 ± 0.98	1.25 ± 0.75	4.08 ± 0.79	1.246 NS	4.92 ± 1.24	0.67 ± 0.65	4.42 ± 0.99	0.804 NS
Emamectin benzoate	4.08 ± 1.24	3.25 ± 1.36	2.67 ± 1.07	0.2055 NS	5.5 ± 1.45	0.83 ± 0.72	3.67 ± 1.30	1.043 NS	5.58 ± 1.44	0.33 ± 0.49	4.08 ± 1.56	0.77 NS
Neemix	2.75 ± 0.62	3.33 ± 1.15	3.92 ± 1.24	0.2055 NS	0.5 ± 0.67	0.42 ± 0.51	9.08 ± 2.79	4.13*	0.5 ± 0.67	0.42 ± 0.51	9.08 ± 2.79	4.13*
Camphor oil	3.58 ± 0.99	2.58 ± 0.79	3.83 ± 1.12	0.18 NS	2.92 ± 0.67	1.0 ± 0.74	6.08 ± 1.16	2.912*	1.75 ± 0.75	0.67 ± 0.65	7.58 ± 2.51	3.845*
Coriander oil	4.0 ± 1.13	2.5 ± 0.67	3.5 ± 1.45	0.12 NS	3.25 ± 0.87	1.92 ± 0.67	4.83 ± 1.34	1.324 NS	2.5 ± 0.67	0.67 ± 0.89	6.83 ± 1.19	3.231*

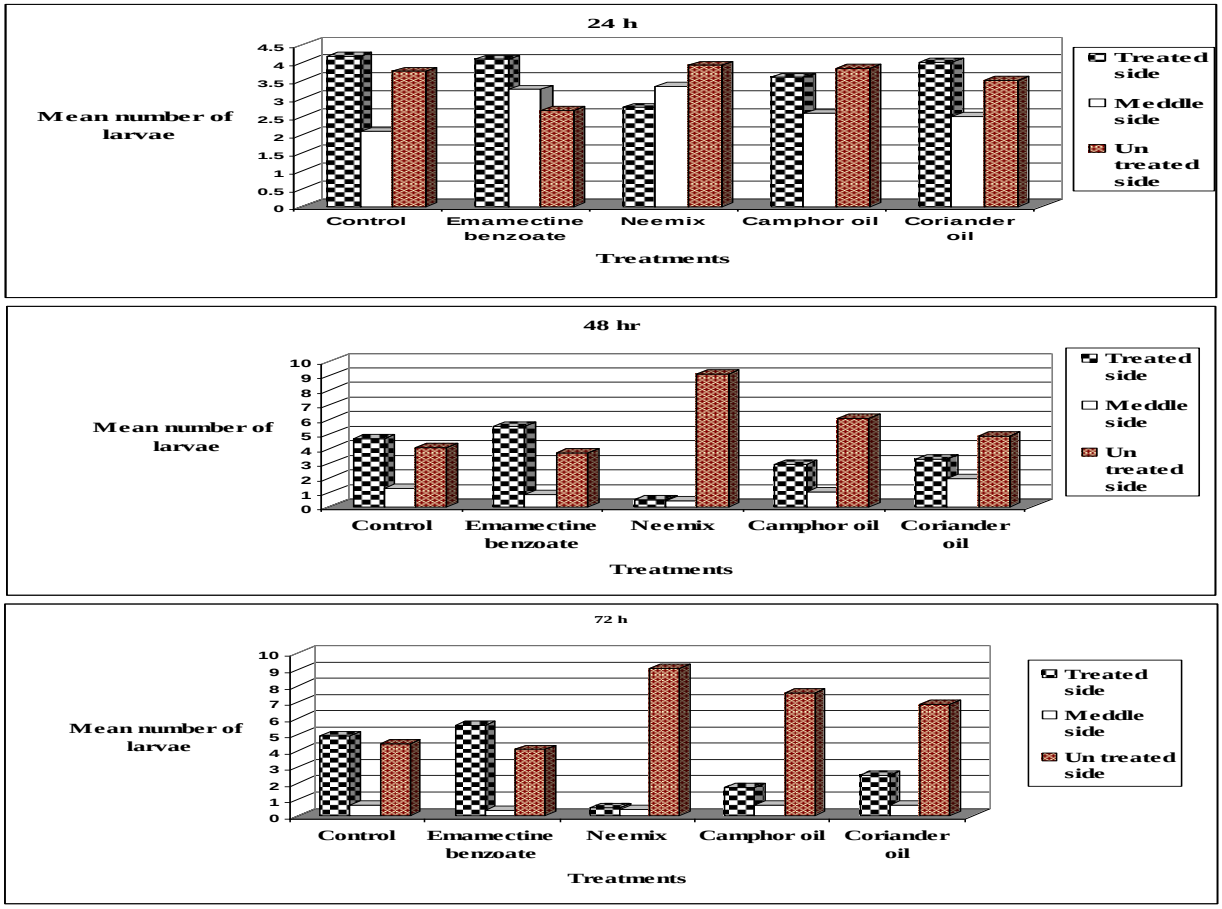

Fig 5. Mean number of of *Hypra brunneipennis* adults stayed in the middle chamber and those oriented to the two treated side chambers having un-treated and treated leaves with the for compounds at concentration corresponding to the 96-hr. LC_{25}.

CONCLUSION

The tested compounds in this research have diverse mode of action and can be included in the IPM module which will very helpful in planning future program of pest management and keep them as part of chemical control to avoid resistance and cross-resistance.

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النشاط الإبادي والطارد لبعض مبيدات الآفات والزيوت النباتية ضد حشرة سوسة ورق البرسيم

علي مصطفي علي

قسم وقاية النبات ، كلية الزراعة ، جامعة المنيا

تعتبر سوسة ورق البرسيم إحدي الآفات الهامة التي تصيب البرسيم في مصر, وفي هذا البحث تم استخدام مبيدين وهما (الإمامكتين بنزوات و النيمكس) واثنين من الزيوت النباتية وهما (زيت الكافور والكزبرة) في مكافحة العمر اليرقي الثاني والحشرات الكاملة لهذه الحشرة تحت ظروف المعمل. تم تقدير التركيز القاتل ل 50 و 90% (LC_{50} α LC_{90}) لهذه المركبات عند فترات 24, 48, 72, 96 ساعة ,وتبعا لقيم (LC_{50} α LC_{90}) وجد أن أعلي المركبات سمية هو الإمامكتين بنزوات يليه النيمكس. من ناحية أخري عند إجراء اختبار الاختيارية مع الحشرات الكاملة باستخدام (LC_{25}) وجد أن أعلي المركبات كفاءة في طرد الحشرات الكاملة كان النيمكس يليه زيت الكافور (حيث كانت النسب المئوية لهذه الحشرة 5 و 17.5% في الجانب المعامل بالترتيب) يليه زيت الكزبرة (25%) بينما المعاملة بالإمامكتين بنزوات كانت أقل معنويا في التأثير الطارد (55.8%) بعد 72 ساعة من المعاملة.