103-112

SINAI Journal of Applied Sciences (ISSN: 2314-6079), Vol.: (2), Is.:(3), Dec. 2013

SINAI Journal of Applied Sciences

JOINT ACTION EFFECT OF SINAI'S FLORA METHANOL EXTRACTS WITH SOME NATURAL OILS ON RICE WEEVIL *SITOPHILUS ORYZAE* L. AND BROAD BEAN BEETLE *BRUCHUS RUFIMANUS*

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ABSTRACT

The research was conducted at the laboratory of Environmental Protection, Faculty of Environmental Agricultural Sciences, El-Arish, Suez Canal University, Egypt, to study the effect of toxicity extracts of methanol to some wild plants in the Sinai (Ader *Artemisia monosperma*, neem *Azadirachta indica*, Lantana *Lantana camara*, nicotine *Nicotiana glauca*) with mixing some natural oils (sesame, castor, flax, camphor, menthe) against some pests of stored grain (rice weevil *Sitophilus oryzae*, broad beans beetle *Bruchus rufimanus*) under laboratory conditions. The results showed that all natural oils have given toxic effects in addition to methanol extracts (polar solvents) for all plants tested against adult insects from the rice weevil and broad beans beetle. Generally, methanol extract of nicotine plant without mixing gave the highest toxicity gainst the adult of rice weevil, while methanol extract of neem plant and mixed with castor oil induced the highest toxicity action against broad bean beetle compared to the other plant extracts under laboratory conditions.

Key words: Methanol extracts, natural plant oils, rice weevil *Sitophilus oryzae* L. and broad bean beetle *Bruchus rufimanus*.

INTRODUCTION

Stored-product insects can cause post-harvest losses, and destroy approximately 5-10% of stored products in developed countries to 20% or more in developing countries **(Marija *et al.,* 2008 and Phillips and Throne, 2010).** Seed stored for more than six weeks must be protected against insect damage. Seed should only be stored when 'dry', as grain of high moisture causes temperatures to rise and mould to develop. High temperatures reduce the efficacy of grain protectants, allowing insects to multiply. Cooling of grain (below 15 degrees C) helps to suppress insect activity **(Pala and Pathipati 2010).** The uses of plant materials in pest control become an important alternative to the use of synthetic insecticides. Plants are a rich source of the chemical compounds with various medicinal and insecticidal properties (**Arnason *et al.,* 1989).** Rice weevil *Sitophilus oryzae* L. and broad bean beetle *Bruchus rufimanus* have been reported as the severe pests of cereal grains and their products (**Baloch, 1992 and Abdul Majeed 2011).** The pest prefers soft varieties of wheat grains **(Zakladnoi and Retanova, 1987).** Attempts have been made to get complete control of the stored grain insects by insecticidal application but in vain. Moreover, fumigation is the most widely adopted method and has been in practice. None of these methods and products can be declared as safe to the precious lives of human beings, birds, beneficial insects, animals and to the environment **(Metcalf, 1982).**

It shows considerable potential for controlling various post-harvest insect pest species of store products-in particular, weevils (*Sitophilus sp.*), flour beetles (*Tribolium* sp.) and pulse beetles (*Callosobruchus sp.*) **(Sanguanpong, 1996).** Furthermore many authors demonstrated the effectiveness of such plant oils in protecting grains from store insects i.e. sesame oil **(Senguttavan *et al.*, 1995);** soybean and castor oil **(Pacheco *et al.*, 1995**; **Senguttavan *et al.*, 1995);** sunflower and rape seed oil **(Tembo and Murfitt, 1995).**

Various essential oils are among the most widely used materials in store pest control, particularly *Acorus calamus* oil **(El-Nahal *et al.*, 1989**; **Schmidt and Streloke, 1994);** *Lantana camara* oil **(Saxena *et al.*, 1992)** including some volatile substances as camphor, α-pinene, linalool and eugenol **(Regnault-Roger and Hamraoui, 1995).**

The present study aimed to investigate the joint action effects of some plant extracts mixed with some natural oils against rice weevil *Sitophilus oryzae* and broad bean beetle *Bruchus rufimanus* in laboratory.

MATERIALS AND METHODS

This experiment was conducted at the Laboratory of Environmental Protection, Department of Environmental Protection, Faculty of Environmental Agricultural Sciences, El-Arish, Suez Canal University, North Sinai Governorate, Egypt, to study the toxicological effect of some Sinai's Flora *Artemisia monosperma*, *Azadirachta indica*, *Lantana camara* and *Nicotiana glauca,* and different plant oils from sesame, castor, linseed (flax), menthe and camphor against rice weevil *Sitophilus oryzae* and broad bean beetle *Bruchus rufimanus* under laboratory conditions.

Test plant material:

Fresh plants of Ader (*Artemisia monosperma*), Neem (*Azadirachta indica*), *Lantana camara* and Tobacco tree (*Nicotiana glauca*) were collected from the El-Maghara and El-Arish regions, North Sinai Governorate, Egypt, air-dried in the Glass Greenhouse.

Dried plants were then ground to powder using an electric grinder. Plant extracts were prepared according to the method described by Talukder and Howse (1993) with some modifications. 5 gram powder of whole plant of Ader (*Artemisia monosperma*), and Lantana (*Lantana camara*), leaves and flowers of Tobacco (*Nicotiana glauca*) and fruits of Neem (*Azadirachta indica*) mixed with 100 ml methanol solvent and then left to stand for 24 hours overnight.

The LC_{25}, LC_{50}, LC_{75} and LC_{90} were calculated directly against rice weevil *Sitophilus oryzae* and broad bean beetle *Bruchus rufimanus* after 5th days of exposure. **Tested Oils:** The concentration of plant methanol extracts which killed 50% of tested insects (LC_{50}) and showed considerable efficacy during the first experiment were mixed with five essential oils to observe their additive effects. (Popović *et al.,* 2006). Five tested oils were tested, namely: sesame *(Sesamum indicum)*, castor *(Ricinus communis)*, linseed or flax *(Linum usitatissimum)*, mentha (*Mentha sp.)* and camphor (*Eucalyptus globulus)*oils. The pure tested oils were commercial samples mixed with LC_{50} of plant extracts, and prepared as follows: 5 drops of "TritonX-100 as emulsifier was mixed thoroughly with 5 ml of each tested oil, then plant extract was added to obtain the same concentration in percent of (v/w).

The emulsifier was mixed at the corresponding concentrations and used as check. the data about the multiplication of insects inside the experimental units and mortality thereof were recorded at five days **(Sabbour and Abd-El-Aziz 2007).**

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Insect rearing:

1. Rice weevil *Sitophilus oryzae* L.:

Colonies of rice weevil, *Sitophilus oryzae* were reared on whole rice grains *Oryza sativa* initially 13 to 14% moisture content (Chen, 2003) in plastic container under the laboratory conditions at 28 ± 2°C, 75 ± 5% R.H. and L12:D12 (Rahman and Talukder, 2006). The subcultures and the tests were carried out under the same conditions. 7 to 14 days old adult of *S. oryzae* were used to the experiments.

2. Broad bean beetle *Bruchus rufimanus* (Boh.):

The broad bean beetle, *Bruchus rufimanus* (Boh.) were reared according to the method described by Sabbour and Abd-El-Aziz (2007). The broad bean beetle were reared on broad bean seeds *Vicia faba* (L.) in plastic pots (20 x 20 x 17 cm) under laboratory conditions 28 ± 2 °C and 75 ± 5% R.H. Each pot was covered with muslin cloth to avoid escaping of adult beetles.

Data analysis:

For all treatments, the percentage of mortality was plotted against the different extract concentrations, then the data were corrected using Abbott's formula **(Abbot 1925)** for the mortalities in the controls. Probit analysis was done to calculate lethal concentrations LC_{25}, LC_{50}, LC_{75} and LC_{90} and slope of the extract against two insects using the software **(Bakr 2007) Ldp Line.**

RESULTS AND DISCUSSION

1. Toxicity effects of Sinai's Flora extracts and essential oils against rice weevil *S. oryzae* L.:

Data given in Table (1) indicated that joint action effect of methanol extracts with oil plants on rice weevil *S. oryzae* L. of *Artemisia monosperma* at different concentrations at LC_{25}, LC_{50}, LC_{75}, and LC_{90} values. Methanol extracts of *Artemisia monosperma* mixed with menthe oil gave the highest toxicity rates against rice weevil based on LC_{50} value.

The order of LC_{50} of the plant extracts on *S. oryzae* after 5th days, followed by methanol extract of *Artemisia monosperma* mixed with castor oil, respectively. While, methanol extract of *Artemisia monosperma* alone showed low toxicity action against adults of rice weevil *S. oryzae*. Concerning, joint action effect of methanol *Azadirachta indica* extracts mixed with different natural oils after 5th days against adult of *S. oryzae* (Table 1).

Data showed that the increasing efficacy of *Azadirachta indica* extract was correlated with increasing of flax oil. It's clear that methanol extract of *Azadirachta indica* mixed with flax or linseed oil achieved acute toxicity against the adult of rice weevil *Sitophilus oryzae* after 5th days of exposure, followed by methanol extract of *Azadirachta indica* mixed with camphor oil. On the other hand, methanol extract of *Azadirachta indica* only gave the lowest toxicity rates against the adult of *S. oryzae* based on Co-toxic factor. Data represented in same table demonstrated that LC_{25}, LC_{50}, LC_{75}, and LC_{90} values as well as Co-toxic factor of methanol extracts of *Lantana camara* against adult of rice weevil *S. oryzae*. It's clear that the highest toxicity was achieved when adults of rice weevil *S. oryzae* treated with methanol extract of *Lantana camara* mixed with castor oil, followed by methanol extract of *Lantana camara* mixed with sesame oil at LC_{25}, LC_{50}, LC_{75} and LC_{90} levels, respectively.

Whereas, the lowest toxicity rate was obtained when adults of rice weevil *S. oryzae* treated with methanol extract of *L. camara* mixed with flax or linseed oil.

Data in Table (1) showed that joint action effect of methanol extracts of *Nicotiana gluca* mixed with different natural oils against rice weevil *S. oryzae*. Data revealed that methanol extracts of *Nicotiana gluca* alone gave the highest

toxicity rates against rice weevil *S. oryzae* based on LC_{50} value and Co-toxic factor, followed by methanol extract of *Nicotiana gluca* mixed with sesame oil at LC_{25}, LC_{50}, LC_{75} and LC_{90} levels, respectively.

The order of LC_{50} of the plant extracts on *S. oryzae* after 5th days. While, methanol extract of *Nicotiana gluca* mixed with menthe oil showed low toxicity action against adults of rice weevil *S. oryzae*. Similar results were reported by **Hanem (2012).**

The results showed that decreasing of mortality percentage on adult of rice weevil may explain the occurrence of the antagonism effect between the methanol extract of nicotine *Nicotiana gluca* and various natural oils.

2. Toxicity effects of Sinai's Flora extracts and essential oils against broad bean beetle *B. rufimanus*:

The mortality percentage and LC_{25}, LC_{50}, LC_{75} and LC_{90} values as well as Co-toxic factor of *Artemisia monosperma* methanol extracts without and with adding natural oils against adults of broad bean beetle *Bruchus rufimanus* after 5th days of exposure were represented in Table (2).

Results revealed that the highest toxicity action was obtained when adults of broad bean beetle *B. rufimanus* treated with *Artemisia monosperma* methanol extracts when combined with menthe oil, followed by methanol extract mixed with castor oil.

On the contrary, the lowest toxicity of extracts when adults treated with *Artemisia monosperma* methanol extract alone. Concerning, different joint action effect of *Azadirachta indica* methanol extracts with or without adding oil plants against adult of broad bean beetle *B. rufimanus* after 5th days of exposure (Table 2).

It's clear that the highest toxicity was achieved when adults of *B. rufimanus* treated with methanol extract of *Azadirachta indica* mixed with castor oil, followed by methanol extract of *Azadirachta indica* alone at LC_{25}, LC_{50}, LC_{75} and LC_{90} levels, respectively. Whereas, the lowest toxicity was obtained when adults of *B. rufimanus* treated with methanol extract of *Azadirachta indica* mixed with flax or linseed oil.

The LC_{50} values and Co-toxic factor at 5th days exposure to different methanol extracts of *Lantana camara* against adult of *B. rufimanus* are given in Table (2).

Data showed that methanol extract of *L. camara* mixed with menthe oil was most effective extracts of toxicity as compared with other extracts at LC_{50} value and Co-toxic factor, followed by methanol extract of *L. camara* mixed with sesame oil at LC_{50} level and Co-toxic factor, respectively.

On the other hand, methanol extracts of *L. camara* mixed with flax or linseed oil gave low toxicity value against *B. rufimanus* after 5th days of exposure.

Data represented in the same table elucidated that LC_{25}, LC_{50}, LC_{75}, and LC_{90} as well as Co-toxic factor of methanol extracts of *Nicotiana gluca* mixed with different natural oils against adult of broad bean beetle ***B. rufimanus***.

The highest toxicity action was achieved when adults of ***B. rufimanus*** treated with methanol extract of *Nicotiana gluca* alone, followed by methanol extract of *Nicotiana gluca* mixed with sesame oil at LC_{25} , LC_{50}, LC_{75} and LC_{90} levels as well as co-toxic factor, respectively.

Whereas, the lowest toxicity was obtained when adults of ***B. rufimanus*** treated with methanol extract of *Nicotiana gluca* mixed with flax or linseed oil after 5th days of exposure.

The previous results are in agreement with **Mamun, *et al.,* (2009).** They found that acetone, methanol and water extracts

Table (1): Joint action effect of methanol extracts of Sinai's Flora mixed with different natural oils against rice weevil *Sitophilus oryzae* L.

Essential oil	*Artemisia monosperma*						*Azadirachta indica*						*Lantana camara*						*Nicotiana gluca*					
	LC_{2}	LC_{5}	LC_{7}	LC_{90}	Slope	Co-toxic factor *	LC_{2}	LC_{5}	LC_{7}	LC_{90}	Slope	Co-toxic factor *	LC_{2}	LC_{5}	LC_{7}	LC_{90}	Slope	Co-toxic factor *	LC_{2}	LC_{5}	LC_{7}	LC_{90}	Slope	Co-toxic factor *
Methanol only	3.572	3.719	3.873	4.016	38.41 +/- 3.71	81.80	3.407	3.666	3.944	4.213	21.20 +/- 2.32	85.52	2.912	3.345	3.843	4.354	11.20 +/- 1.50	92.94	2.314	2.707	3.167	3.648	9.90 +/- 1.37	100.00
M+ Sesame oil	3.023	3.344	3.699	4.050	15.41 +/- 1.88	90.97	3.028	3.368	3.745	4.121	14.62 +/- 1.32	93.08	2.869	3.197	3.562	3.926	14.35 +/- 1.19	97.25	2.463	2.859	3.318	3.794	10.43 +/- 0.936	94.68
M+ Castor oil	2.747	3.080	3.454	3.829	13.56 +/- 1.04	98.77	3.079	3.396	3.745	4.090	15.88 +/- 1.39	92.31	2.762	3.109	3.501	3.896	13.09 +/- 1.11	100.00	2.692	3.029	3.409	3.791	13.16 +/- 1.04	89.37
M+ Camphor oil	2.785	3.150	3.564	3.948	12.59 +/- 0.94	96.57	2.913	3.261	3.651	4.041	13.84 +/- 1.17	96.14	2.944	3.350	3.811	4.280	12.04 +/- 1.58	92.81	2.564	2.938	3.367	3.806	11.41 +/- 1.10	92.14
M+ Flax oil	3.232	3.461	3.707	3.943	22.64 +/- 1.88	87.89	2.802	3.135	3.507	3.880	13.76 +/- 1.66	100.00	3.513	3.805	4.122	4.430	19.42 +/- 2.05	81.71	3.141	3.456	3.803	4.144	16.25 +/- 1.38	78.33
M+ Menthe oil	2.732	3.042	3.388	3.733	14.42 +/- 1.19	100.00	4.158	4.585	5.056	5.520	15.90 +/- 2.37	68.38	3.406	3.706	4.033	4.353	18.36 +/- 1.97	83.89	3.826	4.157	4.516	4.865	18.75 +/- 2.06	65.12

* Co-toxic factor (%) = (low LC_{50} / Other LC_{50})× 100

Table (2): Joint action effect of methanol extracts of Sinai's Flora mixed with different natural oils against broad bean beetle *Bruchus rufimanus*.

Essential oil	*Artemisia monosperma*						*Azadirachta indica*						*Lantana camara*						*Nicotiana gluca*					
	LC_{25}	LC_{50}	LC_{75}	LC_{90}	Slope	Co-toxic factor *	LC_{25}	LC_{50}	LC_{75}	LC_{90}	Slope	Co-toxic factor *	LC_{25}	LC_{50}	LC_{75}	LC_{90}	Slope	Co-toxic factor *	LC_{25}	LC_{50}	LC_{75}	LC_{90}	Slope	Co-toxic factor *
Methanol only	3.345	3.731	4.161	4.591	14.23 -/- 1.86	87.43	2.853	3.321	3.867	4.435	10.21 -/- 1.13	91.36	3.512	3.982	4.514	5.054	12.38 +/-1.77	81.44	3.693	3.967	4.263	4.547	21.64 +/-2.11	88.43
M+ Sesame oil	3.055	3.471	3.944	4.424	12.17 +/-1.17	93.98	3.024	3.429	3.889	4.355	12.34 -/- 1.19	88.48	2.911	3.387	3.941	4.512	10.26 +/-1.53	95.75	2.871	3.278	3.742	4.215	11.73 +/-1.59	100.00
M+ Castor oil	2.928	3.423	4.001	4.605	).94 +/- 1.10	95.30	2.567	3.034	3.586	4.168	).30 +/- 0.73	100.00	3.417	3.748	4.110	4.466	16.83 +/-1.96	86.53	3.100	3.481	3.910	4.340	13.39 +/-1.22	94.17
M+ Camphor oil	3.372	3.652	3.954	4.247	19.52 +/-2.22	89.32	3.135	3.577	4.082	4.596	11.77 -/- 1.14	84.82	3.072	3.478	3.939	4.405	12.50 +/-1.23	93.24	3.353	3.726	4.142	4.555	14.70 +/-1.88	87.98
M+ Flax oil	3.183	3.617	4.111	4.612	12.14 +/-1.16	90.19	3.344	3.674	4.035	4.391	16.54 +/-1.79	82.58	3.689	4.189	4.758	5.336	12.20 +/-1.80	77.42	3.939	4.355	4.816	5.272	15.46 +/-2.04	75.27
M+ Menthe oil	2.944	3.262	3.621	3.978	14.86 +/-1.41	100.00	2.995	3.342	3.729	4.115	14.18 -/- 1.31	90.78	2.867	3.243	3.668	4.099	12.60 +/-1.28	100.00	3.156	3.508	3.899	4.289	14.68 +/-1.20	93.44

* Co-toxic factor (%) = (low LC_{50} / Other LC_{50})× 100

of *Azadirachta indica* showed a direct toxic effect on red flour beetle.

Among them, Neem seed extract showed the highest toxic effect. The effectiveness of *Azadirachta indica* plant extracts increased proportionally with the increase of doses and decreased with time.

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SINAI Journal Applied Sciences (ISSN: 2314-6079), Vol.: (2), Is.: (3), Dec. 2013

الملخص العربى

التأثير المتداخل لبعض مستخلصات نباتات سيناء البرية مع بعض الزيوت الطبيعية على سوسة الأرز وخنفساء الفول الكبيرة

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أجري البحث بمعمل حماية البيئة بكلية العلوم الزراعية البيئية بالعريش، جامعة قناة السويس، مصر لدراسة تأثير السمية لمستخلصات الميثانول لبعض النباتات البرية في سيناء (العادر، النيم، اللانتانا، النيكوتين) مع الخلط ببعض الزيوت الطبيعية (السمسم، الخروع، الكتان، الكافور، النعناع) على بعض آفات الحبوب المخزونة (سوسة الأرز، خنفساء الفول الكبيرة) تحت ظروف المعمل، وأظهرت النتائج أن جميع الزيوت المخلوطة قد أعطت تأثيراً سمياً مضافاً لمستخلصات الميثانول (المذيبات القطبية) لجميع النباتات المختبرة ضد الحشرات البالغة من سوسة الأرز وخنفساء الفول الكبيرة، وأوضحت النتائج ما يلي:

أولاً: حشرة سوسة الأرز:

أعطى مستخلص الميثانول لنبات العادر مع الخلط بزيت النعناع أعلى سمية ضد الحشرة المختبرة، في حين أعطى مستخلص الميثانول لنبات النيم مع الخلط بزيت الكتان أعطى أعلى سمية ضد الحشرة المختبرة، بينما عند الخلط ما بين مستخلص الميثانول لنبات اللانتانا مع الخلط بزيت الخروع أعطى سمية عالية ضد الحشرة البالغة، أما إستخدام مستخلص الميثانول لنبات النيكوتين بدون خلط أعطى أفضل على الإطلاق ضد الحشرة البالغة لحشرة سوسة الأرز.

ثانياً: حشرة خنفساء الفول الكبيرة:

استخدام مستخلص الميثانول لنبات العادر مع الخلط بزيت النعناع أعطى أعلى سمية، في حين أعطى مستخلص الميثانول لنبات اللانتانا مع الخلط بزيت النعناع أعطى أفضل تأثيراً قاتلاً ضد حشرة خنفساء الفول الكبيرة، بينما إستخدام مستخلص الميثانول لنبات النيكوتين مع الخلط بزيت السمسم أعطى أيضاً أفضل النتائج ضد حشرة خنفساء الفول الكبيرة، وعموماً أعطى نبات النيم أفضل التركيزات القاتلة عند الخلط بزيت الخروع ضد حشرة خنفساء الفول الكبيرة مقارنة بباقي النباتات المختبرة الأخرى تحت ظروف المعمل.

الكلمات الاسترشادية: العادر، النيم، اللانتانا، النيكوتين، حشرة سوسة الأرز، حشرة خنفساء الفول الكبيرة.