LABORATORY EVALUATION OF BIO INSECTICIDES EFFICACY AGAINST LEAF MINER *LIRIOMYZA TRIFOLII* (BURGESS) ON COMMON BEAN PLANTS, *PHASEOLUS VULGARIS L.*

OMAR, H.I.H.[1], M.A.L ABBASSY[2], MAHA A.M.TANTAWY[1] AND WALAA A.A.YONES[1]

1. *Plant Protection Res. Inst. Department of Vegetable Pest Res., ARC, Dokky Giza*
2. *Dep. of Pest Control & Environ. Prot. Fac. of Agric., Damanhour, Alex. University*

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Abstract

A laboratory study was conducled in agric. Res. Station, Etay Elbaroud, Elbehara Gavornorate A.R.C. during 2004 season, to examine the extent of mortality in *L.trifolii (Burgess)* (Diptera - Agromyzidae) larvae exposed to some new groups of bio insecticides using common bean as host plants . The treatments were garlic volatile oil (*Allium sativum* linn) and volatile oil of anise(*Pimpinella anisum*)(.06,.12,.25,.5,.75, and 1.01) compared with spinosad (Tracer 24% WG) (0.006 ,.012 , .025 , .04 , and .05) . Also , the joint action of each volatile oil (garelic or anise oil) with the **bio insecticides** spinosad were carried out against the larvae of leaf miner. Spinosad was more toxic to .*L. trifolii* larvae (LC_{50}=0.011% and LC_{95}=0.165%) followed by anise oil (LC_{50}=0.08% and LC_{95}=0.535%)and garlic oil (LC_{50}=0.169% andLC_{95}=3.087%) respectively. Each of garlic and anise essential oil synergized spinosad with co-toxicity factors of +24.85 and +31.59 , respectively . Results strongly indicated that using the anise and garlic oils as a botenical **bio insecticides** can be effective IPM strategies for reducing of leaf miner populations

INTRODUCTION

The broad bean leaf miner , *liromyza trifolii* (Burgess) is one of the most important insect pest which attack common bean plant in Egypt (Omar and Faris 2000). Biological control of this pest has limited success (Harris et al., 1990) . A result of intensive chemical use , *L.trifolii* has developed resistance to all classes of registered insecticides (Cox et al ., 1995). Thus , new products with favorite environmental characteristics are required for the management of this pest .A laboratory study of mortality in *L trifolii* exposed to some new group of insecticides using common bean host plants was conducted by several investigators (Tokumaru et al ., 2005) who found that spinosad was more toxic to the *L. trifolii* . and reduced the number of feeding and oviposition punctures made by female adults of *L. trifolii* .Also the insecticide action of leaves and seed extracts on the leaf miner mortality are reported by several investigators (Saradhi and Patnaik, 2006 and Ahmed et al.,2007). The objective of this study was conducted to assess the toxicoty of isolated anise and garlic oils on the larvae of *L.trifolii* .The bio insecticide spinosad was used as standard. In addition the joint effect of anise and garlic oil on toxicity of spinosad in *L.trifolii* larvae was tested .

MATERIALS AND METHODS

A-Bio -insecticides used

1- Spinosad , Tracer 24% WG

2- Garlic volatile oil : Garlic volatile oil was isolated from the macerated cloves of garlic, *Allium sativum* . Linn . Batches of garlic gloves were macerated and reconstituted by soaking over night in distilled water and then thoroughly mixed in blender. The mixture was steam distilled using steam distillation apparatus connected with oil clavenger trap (Gunther ,1952) . the oily layer was separated and shaken with execess of anhydrous diethyl ether in a separatory funnel. The ether layer was dried with anhydrous sodium sulfate. The solvent was completely removed in vaccu using a rotary evaporator. The resulting oil was stored in a dark bottle at 4 C until used .

3- Anise volatile oil : the volatile oil anise , *Pimpinella anisum* , was isolated by the same distillation procedure which was described above , using batches of powdered seeds of anise.

B-Bioassay Test

Common bean leaves infested with the leaf miner , *Liriomyza trifolii* (Burgess), were collected from the experimental farm Agriculture Research Station , El-behera Governorate, and identifies in Biological Control Department research, Plant Protection research Institute. The infested leaves were used in different bioassay tests as a field strain according to (Sardhi and Pafnaik 2006). The insecticide activity of the bio insecticide , spinosad the two botanicals , anise and garlic essential oils were studied . Different series of concentrations of each compound were prepared in water . Five petri dishes each with one bean leaf infested with the 2nd and 3rd instars larvae of leaf miner , *L trifolii* , were treated with each concentration of each compound by dipping technique, and kept in incubator under control condition (27 c° & 73% RH) . The number of larvae in each dish were counted before and after seven days from application and recorded to count the larvae mortality. Log concentration – probability regression lines were fitted . Statistical parameters of these lines and different lethal concentration (LC_{25} , LC_{50} and LC_{95}) were determined from the curves using the statically method of (Litchfield and Wilcoxon 1949).The joint action of each volatile oil garlic or anise oil with the bioinsecticide spinosad were carried out against the larvae of leaf miner *L trifolii* , after the determination of the expected LC_{25} value of each essential oil and spinosad .A binary mixture of each volatile oil with spinosad was prepared at the ratio of LC_{25}:LC_{25} from each substance.Thus , 50% mortality was expected to result when the mixture was used .

The toxicity of expected LC_{25} value of each substance was tested to the actual percentage mortality. Therefore , the expected mortality for each binary mixture was

the summation of the actual mortlities . The insecticidal activity of each binary mixture was tested against leaf miner larvae as mentioned previously .

A co toxicity factor which was adopted by (Abbassy et-al.,1979) was taken as a criterion for the evolution of the joint toxin effect of the different pairs of toxicants as follow :

$$\text{Co-toxicity Factor} = \frac{\text{\% observed mortality -\%expected mortality}}{\text{-\% expected mortality}} \times 100$$

This factor was used to differentiate the results into three categories . A positive factor of (+ 20) or more means potentiation , a negative factor of (- 20) or less means antagonism and any intermediate value (between – 20 and + 20) was considered additive effect.

RESULTS AND DISCUSSION

Toxicity of spinosed 24 % W G , and the two isolated oils of garlic and anise to *liriomyza trifolii* (Burgess) Larvae was determined .Table (1) presents results of the toxicity of the natural insecticide spinosad to the larvae of leaf miner *L . trifolii* . The concentrations ranging from 0.0062 to 0.05% produced insect mortalities from 32.1 to 83.9 % .The lethal concentration regression line representing the toxicity of spinosad is shown in figure (1). The LC_{50} and LC_{95} values their confidence limits and slopes are recorded in Table (1). Based On LC_{50} and LC_{95} values , results show that spinosad was more toxic (LC_{50}= 0.011% and LC_{95}=0.165%). This result agree with (Tokumaru et al , 2005). Table (2) presents results of the toxicity of the volatile oils of garlic and anise to the larvae of leaf miner . *L.trifolii* , The concentration ranging from 0.06 to 1.0% of garrlic oil produced insect mortalities from 26.6 to 84.2%. Concerning anise oil concentration ranging from 0.06 to 0.5% produced insect mortalities from 44.4 to 90.5% .Mortality of 100% was obtained by a concentration of 0.75% and above of anise oil The lethal concentration regression lines representing the toxicity of isolated oils are shown in Figure (2) The LC_{50},LC_{95} values ,their confidence limits and slopes are recorded in Table (2) .Based on LC_{50} and LC_{95} values , results show that anise oil (LC_{50}=0.08% and LC_{95}=0.535%) was more toxic than garlic oil (LC_{50}=0.169% and LC_{95}=3.007%) . This result agree with (Saradhi and Patnaik, 2006 and Ahmed et al.,2007)

Results of the joint effect of spionsad and each of garlic and anise oils against the larvae of leaf miner *L.trifolii* are recorded in Table (3). These results show that

each of garlic and anise essential oils synergized spinosad with co-toxicity factors of +24.85 and +31.59 , respectively.This result agree with (Yu *et al* ., 2008)

Table 1. Toxicity of the bio-insecticide spinosad to the larvae of leaf miner , *L.trifolii*

Cncentrate of Spinosad (%) (Traces 24%WG)	%Mortality
0.0062	32.1
0.0125	59.0
0.025	71.0
0.04	72.4
0.05	83.9
LC50(%)	0.01114279
Upper limit	0.01410846
Lower Limit	0.008778394
LC95(%)	0.1652481
Upper limit	0.2951822
Lower Limit	0.09342118
Slope	1.404526

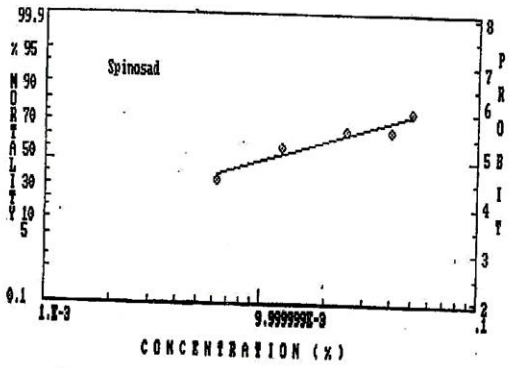

Figure 1. Toxicity of the natural insecticide Spinosad against larvae of leaf miner , *Lirimyza trifolii*

Table 2. Toxicity of Garlic and Anise volatile oils to the larvae of leaf miner ,L.trifolii .

Concentration of Volatile(%)	%Mortality	
	Garlic oil	Anise oils
0.06	26.6	44.4
0.12	40.1	60.3
0.25	66.0	81.7
0.50	70.0	90.5
0.75	80.0	100
1.0	84.2	100
LC50(%)	0.1692924	0.08047006
Upper limit	0.2078211	0.09780616
Lower Limit	0.1361586	0.06563796
LC95(%)	3.007451	0.5358965
Upper limit	5.169647	0.6963377
Lower Limit	1.868051	0.4181884
Slope	1.316382	1.997587

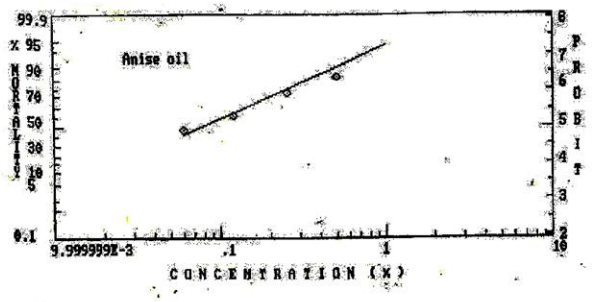

Figure 2. Toxicity of Anise volatile oil against larvae of leaf miner , *liriomyza trifolii* .

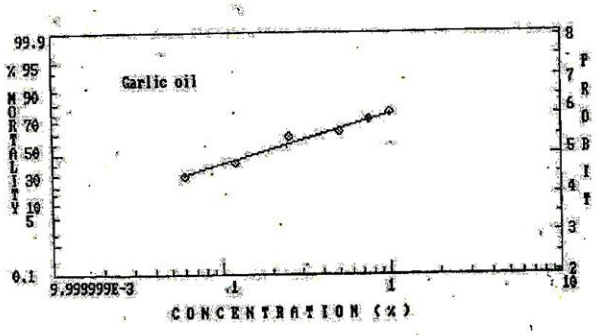

Figure 3. Toxicity of Garlic oil against larvae of leaf miner , *Liriomyza trifolii*

Table 3. Joint action of Garlic and Anise essential oil with bio-insecticide spinosad against larvae of leaf miner , *L.trifolii* .

Volatile oils + *Spiosad*	Calculated % Mortality from treatment LC50 of each toxicant (1)	(2)	Expected mortality (%) (1) + (2)	Observed mortality (%)	Co-toxicity factor
Garlic + Spinosad	12.0	22.2	34.2	42.7	+24.85
Anise+ Spinosad	16.1	22.2	38.3	50.4	+31.59

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تقييم كفاءة بعض المبيدات الحيوية معمليا ضد يرقات صانعات الأنفاق *Liriomyza trifolii* (Burgess) علي نباتات الفاصوليا : *Phaseolus vulgaris (L)*

حافظ إسماعيل حافظ عمر [١] ، مصطفي عبد اللطيف عباس [٢] ،

مها احمد محمود طنطاوى [١] ، ولاء أنور أحمد يونس [١]

١. معهد بحوث وقاية النباتات – قسم بحوث آفات الخضر– مركز البحوث الزراعية – دقي – جيزة .
٢. كلية الزراعة جامعة الإسكندرية – قسم مكافحة الحشرات و حماية البيئة – فرع دمنهور .

أجريت دراسة معملية بمحطة البحوث الزراعية ايتاي البارود ، محافظة البحيرة ، مركز البحوث الزراعية عام ٢٠٠٤ م لاختبار الكفاءة الابادية والتنشيطية للزيوت الطيارة للينسون *Pimpinella anisun* والثوم *Allium salivum* بالمقارنة بالمبيد الحيوي spinosad % WG (Tracer 24)كمركب قياسي ضد يرقات العمر الثاني و الثالث صانعات الأنفاق (Burgess) *Liromyza traiflii* رتبة *Diptera* عائلة *Agromyzidae* التي تم جمعها وتصنيفها من المزرعة التجريبية كسلالة حقلية وكذالك تم دراسة الفعل المشترك الناتج عن خلط زيت الينسون وزيت الثوم مع المركب الحيوى spinosad ضد يرقات العمر الثانى والثالث لصانعات الانفاق . وقد أظهرت الدراسة أن المبيد الحيوي spinosad كان اكثر سمية (LC_{50}=0.011%) من زيت الينسون (LC_{50} 0.08%) ثم يليه زيت الثوم (LC_{50} 0.169%). كذلك أوضحت دراسة التنشيط synergism إلى أن زيت الينسون وزيت الثوم لهما تأثيرا تنشيطيا (زيادة السمية) للمبيد الحيوي spinosadعلي يرقات صانعات الأنفاق حيث كانت قيمة Co-toxicty factor ٣١,٥٩ + ، ٢٤,٨٥+ .هذا يوضح إمكانية استخدام زيت الينسون وزيت الثوم في برامج المكافحة المتكاملة لصانعات الأنفاق .