EFFECT OF SOME VEGETABLE OILS IN ENHANCING THE POTENCY OF BIOINSECTICIDES AGAINST THE COTTON LEAFWORM

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(Manuscript received 2 June 2013)

Abstract

Field investigation revealed that emulsified oils of Cotton seed, Sesame and Soybean enhanced the activity and persistence of the bio-product, Spinosad 24SC, Dipel 2x 6.4 Wp and Protecto 9.4 Wp against the 2nd and 4th instar larvae of the cotton leafworm, *S. littoralis* (Boisd).Physico-chemical properties (Suspensibility, Viscosity and pH value) of tested biocides either alone or in combination with vegetable oils were also determined under field dilution rates (200 Lw/fed).The obtained results clearly showed that ,all the candidate oils were compatible with the tested bio-insecticides, they gave homoganic suspension and increased their viscosity and pH value. Sesame oil scored the highest increase in both viscosity and pH value. The data proved that all the tested oils increased both initial kill and residual activity of candidate biocides. The most evaluated promising synergism, occurred when Sesame oil at 0.3% mixed with full recommended doses of Spinosad, Dipe l2x and Protecto, it showed a remarkable effect in increasing both initial and residual effect after time intervals i.e. zero, 3,7,9 and 14 days post application. It caused 100% larval mortality at the initial time (zero-day) against both 2nd and 4th instar larvae of *S. littoralis*, as compared with biocides alone. Also, residual activity was increased from 49.9, 30.15 and 24.34% to 95.05, 85.13 and 77.25%, respectively against 2nd instar and from 30.15, 12.77and 12.5% to 79.75, 49.15, and 40.4% against 4th instar larvae of *S. littoralis* after 14 days post application. In addition, that all the treated larvae were biologically affected by the three tested biocides alone or in combination with the three vegetable oils. The effect varied according to the larval instar, tested biocide and added oil. Therefore, the treatment of larvae were resulted in decrease the pupation and adult emergence %,pupal weight , adult longevity, fecundity, eggs hatchability and increased the larval and pupal duration and induced the larval,pupal, adult malformations% and shifted the sex ratios% of males and females ,as compared to that of control. The synergistic effect may be due to the addition of vegetable oil that could increase the viscosity of bio-insecticides spray solution. This would increase the deposit on the treated plant leaves, reduce the drift and increase the persistence and improving the target pest control.Also, addition of vegetable oils inhibits respiration and thus, in turn may synergize the toxicity of Spinosad that can act on the nervous system.Also, to change pH of the gut, being more alkaline and thus enhancing the endotoxin breakdown and release of toxic fragments of Dipel 2x and Protecto.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera. Littoralis* (Boisd) was and still now considered one of the most serious phytophagous pests of Egyptian cotton. Larvae attack other wide range of field and horticulture crops such as vegetables, ornamentals and orchards. Intensive use of broad-spectrum chemical insecticides for controlling of *S. littoralis* usually leads to adverse effects on non-target organisms and development of high levels of resistance in pests to organophosphates, carbamates and pyrethroids. The hazards of pesticides and development of resistance to chemical insecticides in the cotton leafworm, *S. littoralis*, necessitates the use of safe and effective insect pathogens such as microbial insecticides.

Microbial insecticides such as *Bacillus thuringiensis* have been reported to provide inadequate control of *S. littoralis* and prime candidates for use in Integrated Pest Management Programme (IPM).They have high pathogenicity for target pests. Safe for most non-target organisms, and have good integration with other pest control methods (Ibrahim *et al.*2010).

Biorational control agents, based on naturally derived products or compounds that disrupt the physiological processes of insects have attracted particular interest.Spinosad is the naturally occurring metabolite derived by fermentation of the actionomycete, *Saccharopolyspora spinosa* Mertz & Yao and it has rapid contact and ingestion activity in insects causing excitation of nervous system leading to cessation of feeding and paralysis. This product is particularly effective against Lepidoptera, Diptera and Thysanoptera. The mode of action of this compound involves the post-synaptic nicotinic acetylcholine receptor and the gama amino btric Acid (GABA) receptor. Spinosad has contact activity on all life stages, as well as stomach poison. It is known that most microbial insecticides have a very short residual activity and the persistence of spores showed an obvious reduction after few days of weathering in Egyptian cotton fields. Degradation of Spinosad in the environment occurs mainly by photo-degradation and microbial degradation (Thompson & Hutchins, 1999). Also most Bt formulations have a very short residual activity, in cotton fields in Egypt has been found that the persistence of spores of B.t showed an obvious reduction after few days of weathering and decouy in its viability was progressively correlated with the time of exposure, because it has a very short effective residual life. The pathogen is not mobile and cannot escape under the unfavorable condition. Ultraviolet radiation seems to be the dominant factor affecting the spores viability (Morris *et al*., 1996). Therefore, the aim of the current study was to investigate how vegetable oils could

enhance the residual activity of tested microbial insedicides for controlling *S. littoralis* under field condition.

MATERIALS AND METHODS

I- Insect: Sufficient egg-masses of cotton leafworm, *S. littoralis* were collected from Sids Agricultural Res.Station, Beni-Sueif Governorate during 2012 cotton season. Larvae were reared under laboratory conditions at 25±2C° and 60±5%R.H. The 2nd and 4th instar larvae were used in all field experiments.

П-Bioinsecticides:

1-Dipel 2x: 6.4%W.P.based on *Bacillus thuringiensis* is subsp. Kurstaki **(32,000IU/mg).**Produced by Abbott laboratories, North Chicago.U.S.A.

2-Protecto:9.4%W.P.based on *Bacillus thuringiensis* is subsp. Kurstaki, it contains 9.4% Lepidopteran active toxin produced by the Plant Protection Res.Inst.A.R.C.

3-Spinosad(Spintor 24%SC) is a natural bio-product formulation of a mixture of two natural metabolites,Spinosyn A (C41H65NO10) and D (C42H67NO10).It was used at a rate of 50cm³/Feddan produced by Dow Agrosciences Chemical Co.

Ш-Vegetable oils:

a-Cotton seed oil, contains about 25% saturated acids, 25% oleic acid and 50% linoleic acid, supplied by Alexandria company for oils and Detergents.

b-Sesame oil, contain 0.4-1.1% Sesamin, 0.3-0.6% Sesamolin and only traces of Sesamol, supplied by EL-SalamCo.for oils.

c-Soybean oil contains about 2.3% Stearic, 8% Vicinoleic and 3.8% Saturated fat, supplied by EL-SalamCo.for oils.

IV-Surfactant agent:

Emulsifier (El-Sisi6) produced by Central Agricultural Pesticides Laboratory, ARC, was used at 0.3%.

V- Physico-chemical properties

Physico-chemical tests were studied for the tested bioinsecticides alone and their combinations with vegetable oils.Suspensibility assay of the tested bioinsecticides alone and their mixtures with oils was carried out to select the best mixture showing the physical compatibility according to WHO specifications (Anonymous, 1979) under field dilution rate (200Lwater/fed).The PH value was measured at 20C° by using PH-meter, While viscosity was measured by using Ostwold viscometer.

VI- Semi-field experiments:

Were performed in cotton fields in Sids Agric.Res.Station, Beni-Suief Governorate during June 2012cotton seasone.To evaluated the initial and residual activity of candidate bioinsecticides alone and their mixtures with vegetable oils, the biocides, Diple 2x, Protecto and Spinosad were applied at their recommended rates 200 gm, 300 gm and 50 cm^3/fed, respectively and their combinations with 0.3%vegetable oils. All crude tested oils were diluted in 0.3%emulsifier (EL-Sisi6) to prepare aqueous emulsions. The treatments were sprayed on cotton plants variety Giza 80 using a solo motor under field dilution at rate (200 Lwater/fed). Samples of treated cotton leaves randomizely collected from each treatment at different time intervals i.e. Zero, 3,7,9 and 14 days after application. Then leaves were transferred to the laboratory and offered to 2^{nd} and 4^{th} instar larvae of *S. littoralis*. Five replicates (ten larvae/replicate) were fed on treated leaves for 48 hrs., then the alive larvae fed on untreated cotton leaves until pupation. Other five replicates were fed on untreated cotton leaves as check. The treated larvae were examined daily to determine the mortality percentages. The larval mortality was recorded and corrected using Abbotts'formula (1925). Different biological aspects such as larval, pupal duration, pupation and adult emergence percentages and adult fecundity, longevity and sex ratio and eggs hatching percentage, larval, pupal and adult malformations were also determined.

VII-Statistical analysis:

The data of the biology were statically calculated through Excel for windows computer program to determine the F-value, P-value and L.S.D (least significant difference at 0.05 or 0.01freedom degrees.

RESULTS AND DISCUSSION

1-Effect of vegetable oils on the physico-chemical properties of tested biorational product:

Physico-chemical properties of Spinosad, Dipel 2x and Protecto (Suspensibility, Viscosity and PHvalue) were measured at total field application volume rate (200LW/fed.) either alone or in combination with the tested plant oils. The results clearly showed that, all the evaluated oils were almost compatible with tested bioinsecticides (Table 1). They gave good suspension without any oil separation or precipitation at the bottom of cylinder after half an hour.Suspensibility was considered as a limiting factor for successful mixtures and would be an important guide for the physical compatibility of the mixed material [Anonymous,1979 WHO specification]. Viscosity of biocides were also increased (Table 1) when they were mixed with 0.3%

vegetable oils + 0.3% emulsifier. Sesame oil gave the highest increase in viscosity 13.59Mps when combined with Spinosad in comparison with Spinosad alone 10.08 Mps, followed by cotton seed oil 13.33 Mps. Also, Sesame oil gave the highest increase in viscosity when mixed with Dipel 2x and Protecto, it gave 11.28 and 13.5 Mps as comparison with Dipel 2x and Protecto alone 10.24 and 10.35 Mps respectively, followed by cotton seed oil 11.2 and 13.33 Mps, respectively. Whereas, Soybean oil revealed the least activity in increasing the viscosity of the tested bioinsecticides. The increase in viscosity of spray solutions would increase the deposit on the treated plant leaf surfaces, reduce the drift and increase the persistence of insecticides[Bode *et al.*,1976].

Table 1. Physico-chemical properties of Spinosad, Dipel 2x, Protecto and their combinations with tested vegetable oils under field dilution rates.

Combination	Rate/Feddan	Precipitation (M1)	Viscosity Mps	PH value
Spinosad 24%Sc	50cm³	0.0	10.08	6.95
S+Cotton seed oil	50cm³+0.3%	0.0	13.33	7.55
S+Sesame oil	50cm³+0.3%	0.0	13.59	7.75
S+Soybean oil	50cm³+0.3%	0.0	12.59	7.21
Dipel 2x 6.4% Wp	200gm	0.0	10.24	6.6
D+ Cotton seed oil	200gm+0.3%	0.0	11.20	7.32
D+ Sesame oil	200gm+0.3%	0.0	11.28	7.26
D+ Soybean oil	200gm+0.3%	0.0	11.15	7.22
Protecto 9.4 Wp	300 gm	0.0	10.35	6.8
P+ Cotton seed oil	300 gm+0.3%	0.0	13.33	7.85
P+ Sesame oil	300 gm+0.3%	0.0	13.5	7.85
P+ Soybean oil	300 gm+0.3%	0.0	11.59	7.80

S: Spinosade

* Mps=Mill poase D: Dipel 2x

P: Protecto

Vegetable oils such as cottonseed or soybean oil were useful for improving the properties of teste bio-insecticides formulations when ultra-low volume application is used for regulating the number of drops and their volume median diameters, which were in many cases playing an important role for increasing the efficacy of insecticides in aerial application. Spray oils increase toxicity, penetration and persistence of contact insecticides and serve as a useful addition to various insect control agents. Vegetable oils may increase the uptake of the toxicant by the insect or reduce its evaporation dissipation or both.

In some insect species, oils inhibit respiration and this, in turn may synergize the toxicity of insecticides that act on the nervous system. Abhilash and Patil (2006) reported that vegetable and mineral oils increased the adhesion, wetting and spreading properties of pesticides on the surface of the targets, decreasing pesticide loss and improving pest control. Also, data in Table (1) showed that PH values of Spinosad, Dipel 2x and Protecto were increased from 6.95,6.6 and 6.8 to 7.55,7.32 and 7.85, respectively, when combined with cotton seed oil and were increased to 7.75,7.26 and 7.85 when mixed with Sesame oil and to 7.21,7.22 and 7.8 with soybean oil.Spinosad is stable at PH ranging between 5-8 (Thompson& Hutchins ,1999). The activity of delta endotoxin of *Bacillus thuringiensis* (Bt) preparation increased as alkalinity increased from8 to10 and then declined rapidly at PH>10 (Gringorten *et al,*1992).

2-The initial and residual effects of the tested bioinsecticides and their combinations with vegetable oils under field conditions.

The initial and residual effect of biorational products alone and their mixtures with 0.3% plant oils at indicating time intervals i.e. zero, 3, 7, 9 and 14 days post application against 2nd and 4th instar, larvae of *S. littoralis* are shown in Table (2). Data revealed that all the evaluated oils increased initial kill and residual activity of tested biocides. Cotton seed, Sesame and Soybean oils at 0.3% increased mortalities when combined with Spinosad,Dipel 2x and Protecto at recommended dose, they caused 100% larval mortalities against 2nd and 4th instar at the initial time interval (zero-day) except with cotton and soybean oils when they mixed with protecto, as compared to bioinsecticides alone 93.6, 87.5 and 80.85%, respectively on 2nd instar and 79.9,65 and 60%, respectively, on 4th instar.Also, tested vegetable oils increased residual activity of tested biorational products after 14 days post application. Mean residual effects of Spinosad, Dipel 2x and Protecto were increased when mixed with 0.3% cotton seed, Sesame and Soybean oils from 49.9,30.15 and 24.34% to 92.5, 95.05,89,76.25,85.13,70,70,77.25 and 59.58%, respectively, against 2nd instar larvae of *S.littoralis* and from 30.15, 12.77 and 12.5% to 75,79.75,68.5,39.28,49.15,32.03,37.4, 40.4 and 31.65, respectively, against 4thinstar larvae of *S. littoralis* as illustrated in figs. 1,2,3,4 and 5.

It could be concluded that the initial kill but residual effect of the tested bioinsecticides markedly increased by using vegetable oils at 0.3% in combination with full recommended doses of Spinosad,Dipel 2x and Protecto.Sesame oil was the most synergistic additive, it showed a remarkable effect in enhancing biocides potency followed by cotton seed oil, while soybean oil came at last in order in enhancing the potency of tested bioinsecticides.The synergistic effects of vegetable oils with

Spinosad,Dipel 2x and Protecto may be due to the addition of such oils which increased the viscosity and PH value of biocides spray solution, this would increased the deposit on the treated plant leaves, reduce the drift and increase the persistence of biocides and protect the spores from UV radiation [Bode et al.,1976].

It is known that most bio-insecticides have short residual activity and persistence of components or spores showed an obvious reduction after few days of weathering in Egyptian cotton fields. Degradation of Spinosad in the environment occurs mainly by photo and microbial degradation (Thompson and Hutchins, 1999).In previous studies, Spinosad showed moderately initial and residual effects 58.7 and 75.95%, when tested single at 50cm/feddan against *S. littoralis*(Abdel Mageed *et al*., 2006).Also, residual activity of Spinosad on cotton plants was up to7 days with 40% mortality on *Agrotis ipsilon* 2nd instar larvae (Mahmoud, 2004).Also, *Bacillus thuringiensis*(Bt) formulations have a very short residual activity ,in cotton fields in Egypt has been found that the persistence of spores showed an obvious reduction after few days of weathering, the pathogen is not mobile and cannot escape under the unfavorable conditions[Moar *et al*.,1986 and Morris *et al*.,1996].In order to increase Bt potency, the conditions prevailing insect midgut must be modified by incorporation of some selected compounds. This compounds must be non-toxic to man or animal, possess no harmful effect on plants, biodegradable and commonly available at low price (EL-Moursy *et al*, 1996).

Vegetable and mineral oils increased the adhesion, wetting and spreading properties of pesticides on the surface of Targets, decreasing pesticides loss and improving pest control (Abhilash and Patil, 2006). Obtained results agree with those of Mahmoud (2004) who reported that Spinosad when mixed with tagetes oil (50 cm/fed +500cm/fed) produced high reduction (90.46 and 82.46%) for pink and spiny bollworms. In addition, Temerak (2003) stated that Spinosad 24%Sc at 50cm/fed when mixed with 1L/fed mineral oil showed best reduction in both cotton bollworm, especially *E.insulana*.Also, it showed 92% reduction in both pink and spiny bollworm as comparison with Spinosad alone 77%.Adding mineral oil enhancing Spinosad performance and was significantly better than Spinosad alone. In addition,Grovena *et al*. (2002)found that Spinosad when mixed at 7.5,10,12.5 and 15ml/100L water + 250ml/100L water of mineral or vegetable oil showed lower % of damaged leaves by *phyllocnistis citrella* after 21 days of spraying.

Table 2. Initial and residual activity of the tested bioinsecticides and their combination with vegetable oils against *S. littoralis* treated as 2nd and 4th instar larvae, under field conditions.

Combination	Larval mortality% after indicating time interval(days)										Mean residual Activity %	
	Initial kill % (zero day)		Residual activity %									
			3 days		7days		9 days		14 days			
	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar	2nd instar	4th instar
Spinosad24SC	93.6	79.90	68	51.66	52.78	40	42.2	22.2	36.6	6.7	49.9	30.2
S+cotton oil	100	100	100	87.5	100	77.5	90	72.5	80	62.5	92.5	75
S+sesame oil	100	100	100	90	100	87.5	92	77.5	88.2	64	95.1	79.8
S+ soybean oil	100	100	100	78	92	75	87.5	65	76.5	56	89	68.5
Dipel 2x6.4Wp	87.5	65	43.48	20	36.96	13.3	10	5	0.0	0.0	30.2	12.8
D+ cotton oil	100	100	87.5	55	80	40	77.5	36.6	60	25.5	76.3	39.3
D+ sesame oil	100	100	92.5	60	90	55	82.5	45	75.5	36.6	85.1	49.2
D+ soybean oil	100	100	80	45	75	36.6	70	26.5	55	20	70	32.0
Protecto9.4Wp	80.85	60	36.96	17.5	28.57	7.5	7.5	0.0	0.0	0.0	24.3	12.5
P+ cotton oil	100	93.6	85	45	77	42.5	62	36.6	56	25.9	70	37.4
P+ sesame oil	100	100	90	50	82	45	73	36.6	64	30	77.3	40.4
P+ soybean oil	100	90	75	40	63.3	36.6	54	30	46	20	59.6	31.7
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

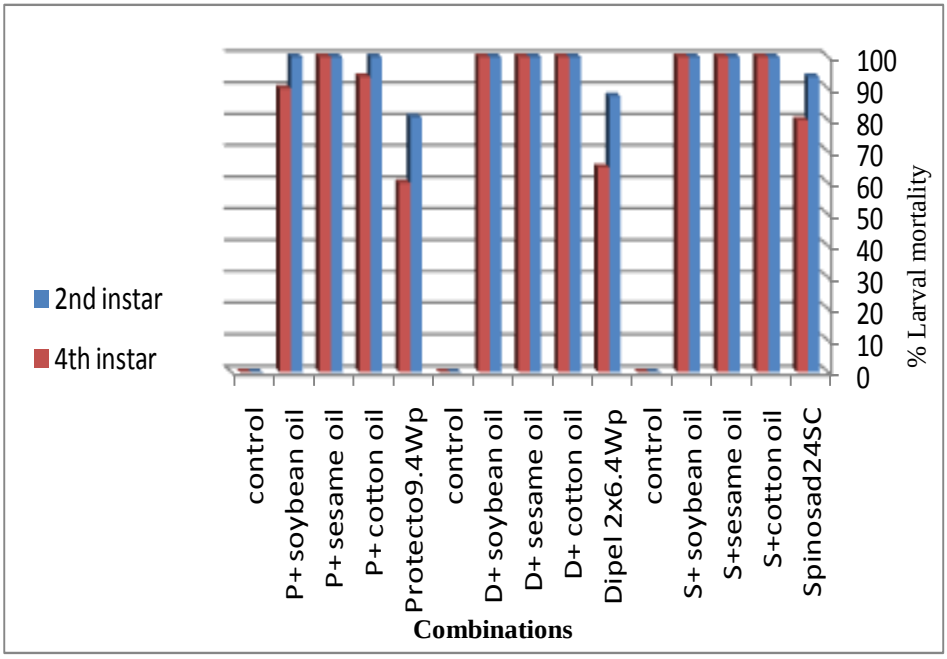


Fig.1. Initial activity (zero time) of the tested bioinsecticides and their combination with vegetable oils against *S. littoralis* treated as 2nd and 4thinstar larvae, under field conditions

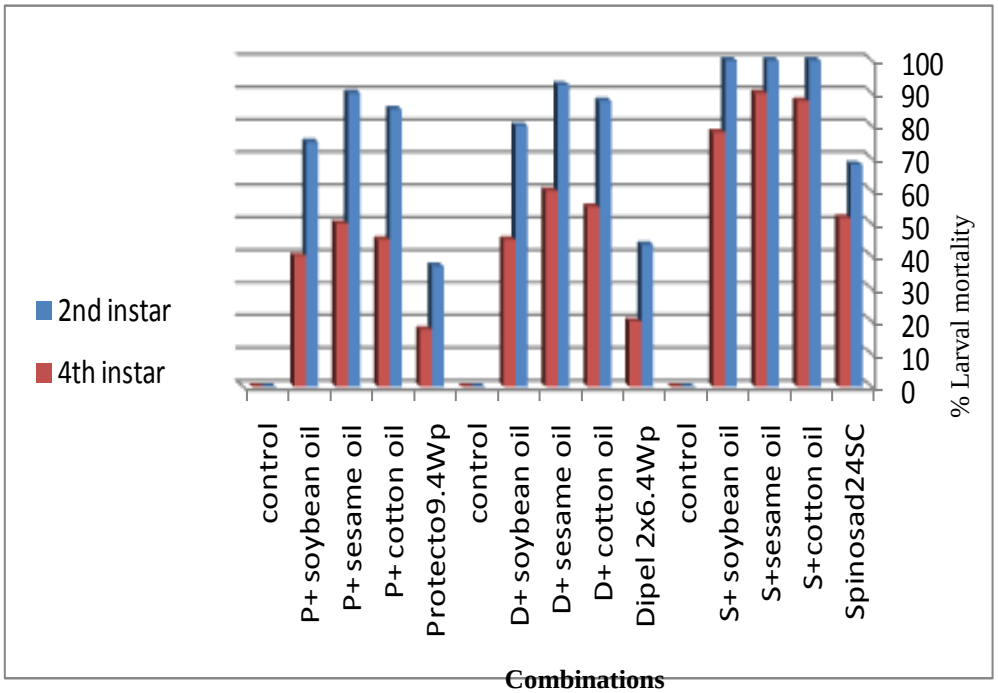


Fig.2. residual activity (after 3days) of the tested bioinsecticides and their combination with vegetable oils against *S. littoralis* treated as 2nd and 4thinstar larvae, under field conditions

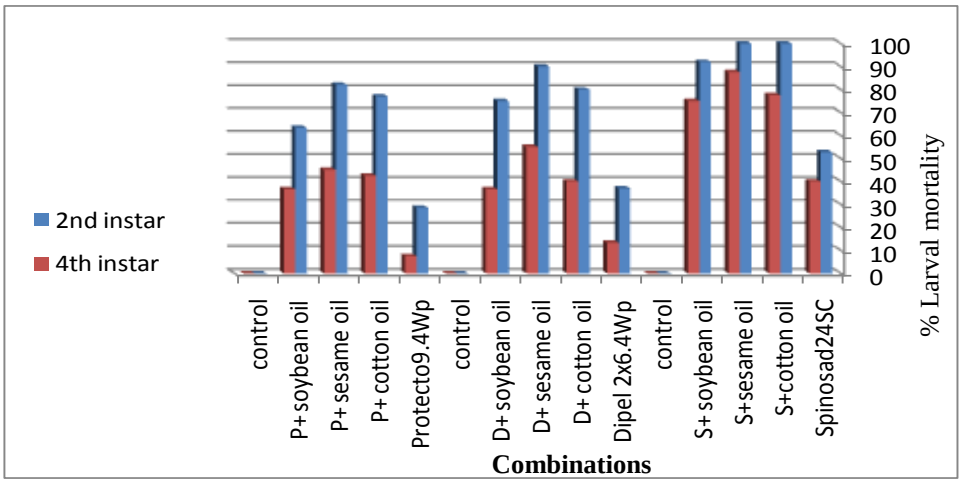


Fig.3. residual activity (after 7days) of the tested bioinsecticides and their combination with vegetable oils against *S. littoralis* treated as 2nd and 4thinstar larvae, under field conditions

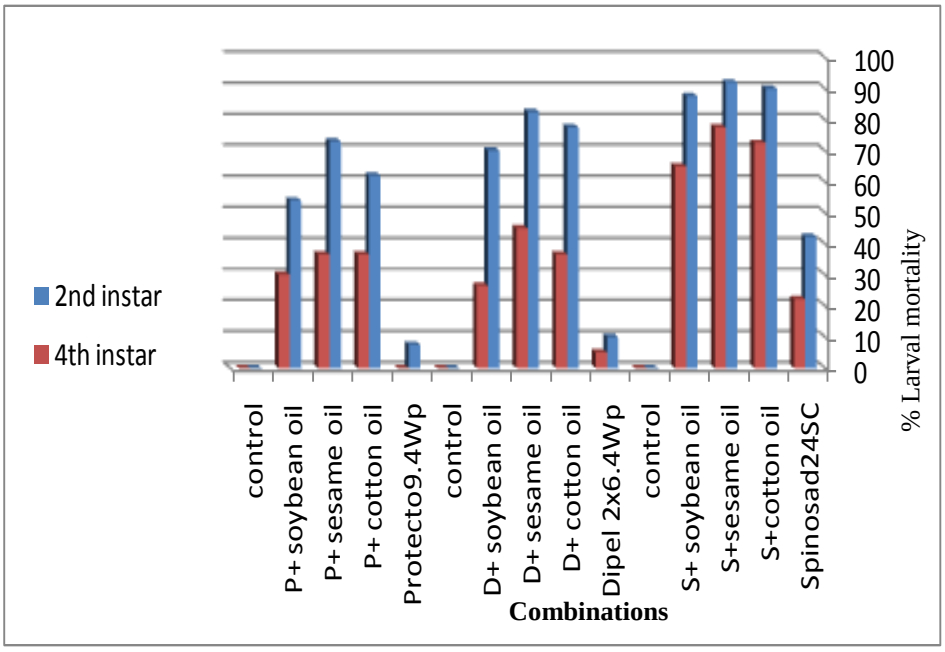


Fig.4. residual activity (after 9days) of the tested bioinsecticides and their combination with vegetable oils against *S. littoralis* treated as 2nd and 4thinstar larvae, under field conditions

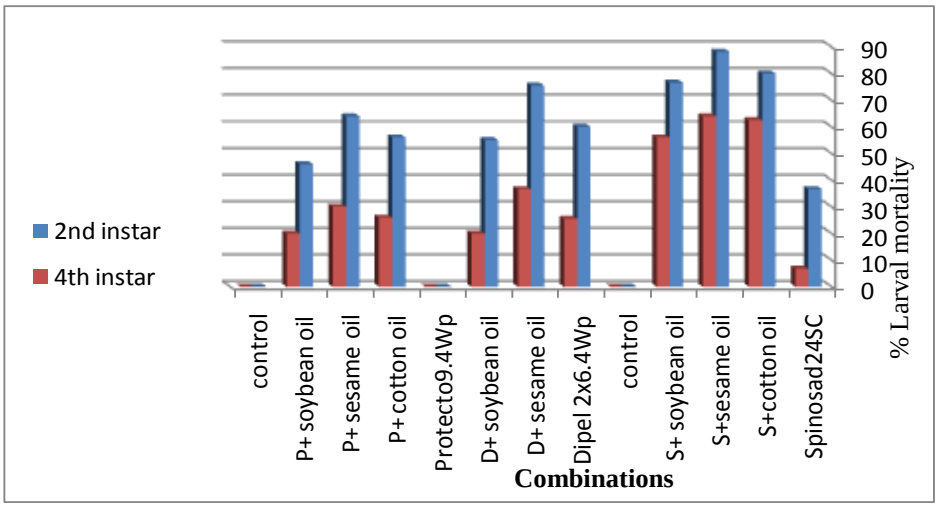


Fig.5. residual activity (after 14days) of the tested bioinsecticides and their combination with vegetable oils against *S. littoralis* treated as 2nd and 4thinstar larvae, under field conditions.

Also, Abdel-Hafez and Abdel-Aziz (2010) indicate that emulsified oils of Tagetes, Sesame and (Soybean+ Sunflower) enhanced the potency and persistence of Spinosad 24Sc against 2ndinstar larvae of *S.littoralis* under laboratory condition. The most promising synergism, occurred when Spinosad was combined with0.25% Targets oil, followed by 0.5%Sesame oil. Combination of Spinosad+Tagetes oil was 3.5, 4.8 and 4.3 times more toxic for the target insect pest than Spinosad alone after 48, 72 and 168hr post exposure.

Formulation containing Soybean, Cotton seed and Corn oils may be useful as bases of spray adjuvant to increase the effectiveness of Bt used to control Tobacco budworm, *Heliothis virescens* and bollworm,*Heliothis zea* (Hafez *et al.*,1987).In addition,Robredo and Obama(1987) stated that using Soybean oil as carrier for Bt in ULV-applications increase the efficacy of the bacterial insecticide from 79.3 to 92.1% against *Thaumetopaea pityocampa*. Temerak (2003) and Turanili *et al*,(2012)found that combination of Dipel 2x 50gm/100 litres+250ml mineral oil gave the highest reduction on potato tuberworm,*Phthorimaea operculella*.Also, combination of Bt var. Kurstaki (Delfin), Bt var aizawi (xentari WG) and neem seed oil gave the highest reduction on *S. littoralis*.

2. Latent effect:

2.1. Larval and pupal durations:

Data presented in Table (3 and 4) demonstrated the residual effect of the three tested compounds (Spinosad, Dipel 2x and Protecto) reflected the biological activities of *S. littoralis.* The feeding of both 2nd and 4th instar larvae on treated cotton leaves after 14 days of the three compounds, increased the larval duration. The effect was more pronounced with the 2nd instar treated with the three compounds mixed sesame oil. The larval duration showed highly significant ($p < 0.01$) increase to average 18.2, 18.1 and 16.6days for the three compounds ,respectively, compared to 13.9days of control

(untreated 2nd instar larvae). Also, the treatment of 4th instar larvae with the same compounds mixed with sesame oil highly significant($p< 0.01$) increase, the larval duration was 16.8, 17.8 and 17 days, as compared to 12.6 days of the check. While 2nd and 4th instar larvae feeding on Spinosad, Dipel 2x and Protecto combined with cotton seed oil at leaf residues aged 14days induced the next significant increase ($p<0.05$) in the larval duration was 16.7, 16.6, 16.9 and 16.5,17.3 and 16.7 days, respectively, compared to 13.9 and 12.6 days of the control. Whereas, the 2nd and 4th instar larvae treated with the three compounds mixed with soybean oil after 14 days revealed the following increase in the larval duration to average 16.4, 16.3, 16.2 and 16.2, 17 and16.4days,respectively.While, the 2nd and 4th instar larvae treated with the spinosad, Dipel 2x and Protecto alone at 14days of the treatment gave less increase of larval duration to average 16,15.8,15 and 16.4,13.9 and 15days,as compared to that of control.

On the other hand feeding the 2nd and 4th instar larvae on the treated leaves after 14 days of spray of the three compounds alone or their mixtures with sesame, cotton seed and soybean oils gave highly significant ($p<0.01$) increase of pupal duration (Table 3 and 4). The effect was more pronounced with the 2nd instar treated with the three compounds combined to sesame oil. It caused highly significant ($p< 0.01$) increase in the pupal duration, while pupal duration was 23, 28.4 and 26.6 days, respectively, compared to 9.9 days of control. In addition, the larval treatment of 4th instar with the same compounds mixed with sesame oil highly significant ($p< 0.01$) increase the pupal duration, it was 22.8, 23.8 and 25.6 days, respectively, compared was found in to 9.4 days of the check. While 2nd and 4th instar larvae fed on Spinosad, Dipel 2x and Protecto combined with cotton seed oil at treated leaves after 14 days of application induced the next significant increase in the pupal duration to 22, 28 and 25.7 and 22.2 ,22.9 and 25.4 days, respectively, in comparison with control (9.9 and 9.4days). Whereas, the 2nd and 4th instar larvae treated with the three compounds mixed with soybean oil after 14 days induced increase in the pupal duration to average 21 ,27.4, 21.3 and 21.2, ,21.6,23.8 days, respectively. While, the 2nd and 4th instar larvae treated with the spinosad, Dipel 2x and protecto alone after 14 days of the treatment gave less or none increase of larval duration to average 19.5, 9, 10.2 and 19.9, 7.8 9.1 days, as compared to that of control.

The obtained results agree with those obtained by Morillo and Notz (2004) found that the duration of the larval and pupal stages and the developmental period from egg to adult of *S. frugiperda* was significantly longer in the lambdacyhalotrin-selected strain and the methomyl-selected strain compared to the control strain, from the first to the last generation. Ahmed (2004) mentioned that the larval period was elongated and the pupal period shorted for the new hatched larvae of pink and spiny bollworms, (Laboratory strain) treated with the higher concentrations of Spinosad when compared with untreated larvae.

Table 3. Latent effect of the tested bioinsecticides and their combination with vegetable oils *against S. littoralis* treated as 2nd instar larvae, under field laboratory conditions.

Treatment	Larval duration (days) Mean±SD	Malfo. larvae %	Pupation %		Pupal Duration (days) Mean±SD	Pupal weight (mg) Mean±SD	Adult emergence %	
			Normal	Malfo.			Normal	Malfo.
Spinosad24SC	16.0±0.4**	0	63.4**	0	19.5±0.5**	260±7.8**	89.8n.s	10.2
S+cotton oil	16.7±2.5**	0	20**	5	22±1.6**	252±6.8**	76.9**	23.1
S+sesame oil	18.2±2.5**	0	11.8**	7.7	23±3.6**	207±47**	62**	38
S+ soybean oil	16.4±0.8**	0	23.5**	3.3	21.0±1.2	258±36**	85.2*	14.9
Dipel 2x6.4Wp	15.8±1.3**	0	100n.s	0	9+1.6n.s	350±46n.s	100n.s	0
D+ cotton oil	16.6±0.9**	0	40**	3.6	28±0.7	242±1.2**	88.9n.s	11.1
D+ sesame oil	18.1±2.3**	13.3	24.5**	4.2	28.4±0.6**	226±45**	80.0**	20
D+ soybean oil	16.3±0.7**	6.7	45**	3.4	27.4+2.2	244±41**	87.5n.s	12.5
Protecto9.4Wp	15.0+2.1**	0	100n.s	0	10.2+1.8n.s	267±8.2**	91.7n.s	8.3
P+ cotton oil	16.9±1.4**	6.7	44**	9.6	25.7±2.7**	248±61**	85.7n.s	14.3
P+ sesame oil	16.6±1.2**	13.3	36**	12.5	26.6±3.0**	232±46**	77.5**	22.5
P+ soybean oil	16.2±0.6**	6.7	54**	6.7	21.3+2	261±34**	86.7n.s	5.6
Control	13.9±1.2	0	100	0	9.9±1.6	365±52	100	0
F value	111.3711		3239.4		2384.2	27.183	118.695	
P value	0.001132		0.0025		0.0002	0.00064	0.00756	
L.S.D at 0.05	0.504167		12.1		0.75	64.3273	8.42	
0.01	0.775833		27.93		1.02	100.7182	15.44	

** = Highly Significant (p<0.01) * Significant (p<0.05)

S.D. =Standard deviation Malfo. = Malformation%

L.S.D. = Least significant difference Lab**.** =Laboratory strain

N. S=none Significant (p>0.05)

Table 4. Latent effect of the tested bioinsecticides and their combination with vegetable oils against S. littoralis treated as4th instar larvae, under field laboratory conditions.

Treatment	Larval duration (days) Mean±SD	Malfo. larvae %	Pupation %		Pupal Duration (days) Mean±SD	Pupal weight (mg) Mean±SD	Adult emergence %	
			Normal	Malfo.			Normal	Malfo.
Spinosad24SC	16.4±1.8**	0	93.3n.s	0	19.9±0.3**	264±20**	97n.s	3
S+cotton oil	16.5±0.9**	0	37.5**	3.6	22.2±1.9**	233±15**	88.5n.s	11.5
S+sesame oil	16.8±1.7**	0	36**	3.6	22.8±4.8**	206±71**	80.7**	19.3
S+ soybean Oil	16.2±0.5**	6.7	44**	3.6	21.2±1.3**	240±21**	96.5n.s	3.5
Dipel2x6.4Wp	13.9±1.8*	0	100n.s	0	7.8+1.1**	357±33n.s	100n.s	0
D+ cotton oil	17.3±3.2**	0	74.5**	0	22.9+1.1**	228±45**	84.9*	15.1
D+ sesame oil	17.8±3.8**	0	63.4**	0	23.8±1.6**	207±56**	76.7**	23.3
D+ soybean oil	17±3.7**	0	80**	0	21.6+1.8**	229±35**	100n.s	0
Protecto9.4Wp	15+2.1**	0	100n.s	0	9.1+1.6n.s	318±25*	93.8n.s	7.2
P+ cotton oil	16.7±2.2**	0	74.5**	9.1	25.4±3.4**	228±22**	91.7n.s	8.4
P+ sesame oil	17±1**	0	70**	13.4	25.6±1.5**	226±46**	80.6**	19.4
P+ soybean oil	16.4±1.3**	0	80**	3.7	23.8+3.1**	282±16**	92.9n.s	7.1
Control	12.6±0.5	0	100	0	9.4±1.4	375±26	100	0
F value	391.3344		2675.4		1121.963	63.58834	22.375	
P value	0.0000013		0.0023		0.0000551	0.003755	0.01822	
L.S.D at 0.05	0.6658		5.325		0.975	45.02917	16.01	
0.01	0.895		12.288		1.325	70.576	36.962	

** = Highly Significant (p<0.01) * Significant (p<0.05)

S.D. =Standard deviation Malfo. = Malformation%

L.S.D. = Least significant difference Lab**.** =Laboratory strain

N. S=none Significant (p>0.05)

2.2. Pupation and Pupal weight:

Data presented in Table (3 and 4) showed that the 2nd and 4th instar larvae of *S. littoralis* fed on treated cotton leaves after 14 days of application with Spinosad, Dipel 2x and Protecto alone or in combination with sesame, cotton seed and soybean oils. There was highly significant ($p < 0.01$) decrease in the pupation percentage in respect to control. The effect was more pronounced with the treatment of the three compounds combined with sesame oil. The pupation percentages were 11.8, 24.5 and 36 %, respectively of 2ndinstar, and it were 36, 63.4 and 70%, respectively, of 4th instar, compared to 100 % of control. Whereas, the larval feeding of the 2nd and 4th instar larvae with treated leaves with the three compounds in combination with cotton seed oil had next effect in pupation decrease to reach 20, 40, 44 and 37.5, 74.5, 74.5 %, respectively.While, the larval treatment of both instars with the three compounds in combination with soybean oil had the least effect on pupation, it decreased the pupation % to 23.5, 45, 54 and 44, 80, 80%, respectively, compared to check (100%). Consequence, the larval treatment of 2nd and 4th instar with Spinosad, Dipel 2x and Protecto caused the lowest effect in pupation decrease to be 63.4, 100,100 and 93.3, 100, 100%, respectively, compared to 100% pupation of control.

Likewise, the larval feeding of 2ndand 4th instar larvae on the treated leaves after 14 days of spray of the three tested compounds highly significantly ($p<0.01$) reaction in the pupal weight was found of the resulted pupae. The 2ndinstar treated with the three compounds combined to sesame oil was the most suppressive one on the pupal weight, it decreased the pupal weight to 207, 226 and 232 mg, respectively, compared to 365 mg of the untreated 2nd instar larvae. Whereas, the larval treatment of 4th instar with the same compounds combined with sesame oil highly significant ($p< 0.01$) increased was obtained in the pupal weight to be 206, 207 and 226 mg, respectively, compared to the control (375mg). While 2nd and 4th instar larvae fed on leaves treated with Spinosad, Dipel 2x and Protecto combined with cotton seed oil at leaf after 14 days of treatment revealed significant decrease in the pupal weight to 252, 242, 248 and 233, 228, 228 mg, respectively, compared to the control (365 and 375 mg). Whereas, the 2nd and 4th instar larvae treated with the three compounds in combination with soybean oil after 14 days induced decrease in the pupal weight to be 258, 244, 261 and 240, 229, 282 mg, respectively. While, the 2nd and 4th instar larvae treated with the spinosad, Dipel 2x and protecto alone after 14 days of the treatment induced low or none decrease of pupal weight to be 260, 350, 267 and 264, 357, 318 mg, compared to control.

The obtained results similar to that obtained by Ahmed (2004), who found that the average of pupation percentages for pink and spiny bollworms gradually decreased with increasing concentrations of the tested compounds (Agerin, Diple 2x Naturalis L, Spinosad) in laboratory and field strains, respectively.

2.3. Moths emergence:

The larval feeding of 2nd instar on treated cotton leaves with spinosad, protecto and Dipel 2x compounds combined to sesame oil after 14days of the application gave highly significant ($p < 0.01$) decrease of the adult emergence to reache 62, 80 and 77.5%, respectively, as compared to 100% of control. While the larval feeding of the 4th instar on spinosad mixed with sesame oil only induced the adult emergence decrease to reach 80.7, 76.7 and 80.6% in comparison the check (100%). Similarly, the larval treatment of 2nd instar with spinosad in combination with cotton oil induced 76.9% , hence, the 4th instar treated with Dipel 2x combined to cotton seed oil and the 2nd instar treated with Spinosad in combination with soybean oil caused significant ($p < 0.05$) decrease in the adult emergence to average 84.9 and 85.2%, respectively, as compared to that of control (100%).

These results are agree to those obtained with Ahmed (2004) who found that adult emergence for pink and spiny bollworms gradually decreased with increasing concentrations of the tested compounds (Agerin , Diple 2x Naturalis L , Spinosad) in laboratory strain.

2.4. Morphogenetic effects:

Data presented in Table (3 and 4) demonstrated that the larval feeding of *S. littoralis* on the leaf residues of three compounds (Spinosad, Protecto and Dipel 2x) induced larval Deformation % compared to control. The larval treatment of the 2ndinstar larvae with both Dipel 2x and Protecto in combination with sesame oil gave the highest percentage of larval malformation reached 13.3%, respectively, as compared to 0% of control. While the 2ndinstar treated with both dipel 2 x and protecto in combination with soybean oil and the larvae treated with Protecto in combination with cotton seed oil induced 6.7%. (Table 3). Also, the treatment of 4thinstar larvae with Spinosad mixed with soybean oil induced the same percentage (6.7 %), (Table 4).

A similar effect demonstrated with the larval feeding of *S. littoralis* on the leaf residues of the three compounds resulted in increase in the pupal deformation percentage, as compared to the control (0%).The larval treatment of the 2nd with spinosad, Protecto and Dipel 2x in combination with sesame oil showed the greatest percentage reached to 7.7, 4.2 and 12.5%, respectively, as compared to 0% of the control. While the larval treatment with the three compounds in combination with

cotton oil induced the next percentage, it reached 5, 3.6 and 9.6%. Whereas, the larval treatment with three compounds combined to soybean oil gave the least percentage reached to3.3, 3.4 and 6.7%.On the other hand, the larval treatment with the three compounds alone did not give any pupal malformation %. While, the larval treatment of 4th with Spinosad in combination with the three vegetable oils induced 3.6% .Whereas, the 4thinstar treated with the three vegetable oils gave more higher reached to 9.1, 13.4 and 3.7%, respectively, as compared o that of control.

Treatment of the 2nd instar larvae with the three vegetable oils separetly or in combination with tested compounds revealed the highest percentage of malformed adults ranged between 11.2-38.23% compared to 0% of control. While the larval treatment of 2ndinstar with Spinosad, Dipel 2x and Protecto in combination with soybean oil gave 14.9, 12.5 and 5.6%, respectively. Also, the 4thinstar treated with the three compounds in combination of sesame and cotton oils induced the greatest percentage of adult malformations reached to19.3, 11.5, 23.3, 15.1, 19.4 and8.4%, respectively.Whereas, the 4thinstar treated with the three compounds in combination with soybean oil induced the least percentage reached to3.5,0,7.1%, respectively, compared to control.

Deformation of *S .littoralis* pupae resulting from the larval treatment of the 2nd and 4th instars with spinosad, protecto and Dipel 2x in the present work mostly appeared as Malformed larvae appearing shortened and black (Fig.1) or Undersized larvae Fig.2 and 3) or larval-pupal intermediates (Fig.4, 5, 6, 7, 8 and 9) or Malformed pupae were unable to cast the last moulting (Fig.10) or Undersized pupae or abnormal pupae in shape (Fig.11, 12 and 13), as compared to normal pupae **(**Fig.14**)**. Moreover, moth malformations showing completely absent of one of pair of wings and strongly malformed body (Fig.15) or Slight malformation in both wings (Fig.16) or Morphological varieties of malformed body and wings (Fig.17, 18 and 19), as compared to normal adults (Fig.20).

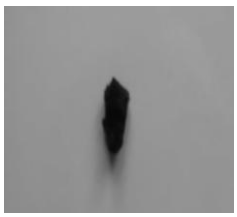
.

(Fig.1): deformed larvae appearing shortened and black

(Fig.2, 3): Undersized larvae

(Fig.4, 5, 6, 7, 8 and9): Larval-pupal intermediates

(Fig.10):deformed pupae were unable to cast the last moulting skin



(Fig.11, 12and13): Undersized pupae or abnormal pupae in shape

(Fig.14) Normal pupae of *S .littoralis*

(Fig.15): Completely absent of one of pair of wings and strongly malformed body.

(Fig.16):Slight malformation in both wings

(Fig.17, 18 and 19): Morphological varieties of malformed body

(Fig.20): Normal adult of *S .littoralis*

These results are agreement to those obtained by Ahmed (2004), who reported that Spinosad gave malformed pupal and adults in both laboratory and field strains of both Pink and Spiny bollworms.

Table 5. Latent effect of the tested bioinsecticides and their combination with vegetable oils against *S. littoralis* treated as 2nd and 4thinstar larvae, under field laboratory conditions

Treatment	Fecundity Eggs/female Mean±SD	Fertility % Eggs/f	Longevity Mean±SD	Adult emergence %	
				Male	female
Spinosad24SC	50±30**	100	11.5±1.7**	50	50
S+cotton oil	zero**	zero	8.3±1.4**	52	48
S+sesame oil	19.3±10**	100	7.7±1.1**	59	41
S+ soybean oil	45±3.8**	100	11.4±1.8**	50	50
Dipel2x6.4Wp	79±21**	100	9.9±0.3**	45.8	54.2
D+ cotton oil	20.2±2.3**	100	8.4±2.3**	56.5	43.5
D+ sesame oil	zero**	zero	8.3±0.6**	54.5	45.8
D+ soybean oil	zero**	zero	9.3±1**	53.3	46.7
Protecto9.4Wp	140±10**	100	10.1±1.5**	47.1	52.9
P+ cotton oil	105±5**	100	8.5±4**	56	44
P+ sesame oil	63±0.7**	100	7.9±2.0**	58.8	41.2
P+ soybean oil	93±7.5**	100	9.6±1.5**	56	44
Control	649±144	100	15.5±1.8	50	50
F value	147.8932		221.6544		
P value	.00707		.000016		
L.S.D at 0.05	191.0955		0.995833		
0.01	441.8282		1.3425		

** = Highly Significant (p<0.01) * Significant (p<0.05)
S.D. =Standard deviation Malfo. = Malformation%
L.S.D. = Least significant difference Lab. =Laboratory strain
N. S=none Significant (p>0.05)

2.5. Adult fecundity and fertility:

Data presented in Table (4and 5) demonstrated that the larval feeding of *S. littoralis* on the leaf residues of the three compounds (Spinosad, Dipel 2x and Protecto), highly significant ($p<0.01$) reduced the adult fecundity in respect of control. The larval treatment of 4th instar with Spinosad combined to cotton seed oil, and Dipel 2x combined with sesame or soybean oils had the strongest effect on the adult fecundity, it completely inhibited the eggs laying (0.0), as compared to 649 eggs/ females of control. While the total number of eggs laid by adult females fed, as4th instar larvae on Spinosad combined to sesame oil and Dipel 2x with cotton oil gave19.3 and 20.2 eggs/ females, respectively, as compared to control. On the other hand, the total number of eggs laid by adult females treated as 4th instar larvae with Spinosad mixed with soybean oil and Protecto with sesame oil or soybean oil had the next effect in adult fecundity reduction to average 45, 63 and 93 eggs/ females, respectively, as compared to that of control (649 eggs/ females).Whereas, the larval

treatment with the three compounds, alone recorded the highest number of eggs(50,79 and 140eggs/female,respectively),as compared to that of control.

Likewise, the larval feeding of *S. littoralis* on the leaf residues of the three compounds (Spinosad, Dipel 2x and Protecto), highly significant ($p<0.01$) reduced the eggs hatching in respect of control. The larval treatment of 4th instar with Spinosad combined to cotton oil, and Dipel 2x combined to sesame or soybean oils had the strongest effect on eggs hatchability, it completely inhibited the eggs laying and hatching (0.0), as compared to 100% of control. While the total number of eggs hatching laid by adult females fed as 4th instar larvae on Spinosad combined to sesame or cotton oil , or Dipel 2x combined to cotton seed oil or Protecto combined to the three vegetable oils didn't' affect on the eggs hatching (100%), as compared to that of control.

2.6. Adult longevity:

Data presented in Table (4 and5) showed that feeding of the 4th instar larvae on the leaf residues aged 14 day sprayed with Spinosad, Dipel 2x and Protecto alone or in combination with the three vegetable oils highly significant ($p<0.01$) decreased the adult longevity of *S. littoralis*.The larval treatment of 4thinstar larvae treated with Spinosad, Dipel 2x and Protecto in combination with sesame oils induced the shortest period of adult longevity to average7.7, 8.3 and 7.9 days, as compared to15.5days of the check.Whereas, the larval treatment with the three compounds combined to cotton oil induced the latent effect, it decreased the adult longevity to average8.3, 8.4 and 8.5days, and the larvae treated with the three compounds in combination with soybean oil had the least effect, it gave 11.4, 9.3 and 9.6days, and it averaged 11.5, 9.9 and10.1days for adult treated as 4th instar with Spinosad, Dipel 2x and Protecto alone, respectively, as compared to that of the check.

2.7. Adult sex ratio:

Data in Table (4 and 5) indicated that the 2nd and 4th instar larvae of *S. littoralis* fed on the leaf residues sprayed with Spinosad, Dipel 2x and Protecto alone or in combination with the three vegetable oils shifted the adult sex ratio in respect to that of the control. It found that the larval treatment of 4th instar with Protecto combined to Sesame oil had the highest effect in adult males increase to reach 58.8%, as compared to 50% of males of control, and decreased the adult females to 41.2%, as compared to 50% of adult females. While, the larval treatment with Protecto combined to both cotton seed and soybean oils had the latent effect in adult males increase to reach 56% and decreased the adult females to reach 44%,as compared to that of control (50:50).On adversely, the larvae treated with the Protecto alone decreased the adult males to reach 47.1% and increase the adult females to reach

52.9%,as compared to control (50:50).Also, the larval treatment of 4th instar with Dipel 2x combined with sesame and cotton oil induced the strongest effect in adult males to reach 54.5 and 56.5%,respectively,and decreased the adult females to reach 45.8 and 43.5% ,respectively.Whereas,the larvae treated with Dipel 2x combined with soybean oil induced the next effect in adult males increase to53.3 and decreased the adult females to 46.7% ,Hence, the larval treatment with Dipel 2x alone decreased the adult male to45.8% ,and increased the adult females to 54.2%,as compared to control. On the other hand, the larval treatment with Spinosad combined to Sesame and cotton oils

induced the highest effect in adult males increase to reach 59 and52%, and decreased adult females 41 and 48%, respectively, Hence, the larval treatment with Spinosad

combined to soybean or Spinosad alone did not affect the sex ratio of both males and females.

2.8. Reviewing the obtained results:

Field studies suggest that, adding vegetable oils enhancing microbial insecticides performance and useful addition for controlling *S. littoralis* reducing the costs of pest control and environmental contamination.

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تأثير بعض الزيوت النباتية في زيادة فعالية المبيدات الحيوية ضد دودة ورق القطن

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معهد بحوث وقاية النباتات – مركز البحوث الزراعية

أجريت التجارب الحقلية لدراسة فعالية الزيوت النباتية: زيت بذرة القطن، السمسم وفول الصويا لزيادة فعالية واستدامة المركبات الحيوية الإسبينوساد، 24SC ، الدايبل ٢ أكس 6.4wp البروتكتو 9.4WP ضد يرقات العمر الثاني والرابع لدودة ورق القطن. أيضاً تم دراسة تأثير الزيوت النباتية على الخواص الطبيعية – الكيماوية [اختبار التعلق – اللزوجة – درجة الأس الهيدروجيني] للمركبات الحيوية تحت التخفيف الحقلي [٢٠٠ لتر ماء / فدان].

أظهرت النتائج بوضوح أن جميع الزيوت مجال الدراسة متوافقة خلطياً مع المبيدات الحيوية حيث أعطت معلق متجانس، هذا بالإضافة إلى زيادة لزوجة ودرجة الحموضة لهذه المركبات وسجل زيت السمسم أعلى زيادة في كلا من اللزوجة ودرجة الحموضة. أيضاً حققت جميع الزيوت النباتية المختبرة زيادة كلا من الأثر الإبادي الفوري والباقي للمركبات الحيوية محل الدراسة كما حقق زيت السمسم أعلى تنشيط وإبادة فورية وأثر باقي عند خلطه بمعدل ٠,٣% مع التركيز الحقلي الكامل لمركب الإسبينوساد، الدابيل، أكس، البروتكتو بعد فترات من الرش [صفر، ٣، ٧، ٩، ١٤ يوم] وأعطى أعلى إبادة فورية بعد الرش مباشرة (صفر يوم) حيث أعطى ١٠٠% موت ليرقات كلا العمر الثاني والرابع لدودة ورق القطن بالمقارنة بالمبيدات الحيوية منفردة، أيضاً أعطى زيت السمسم أعلى أثر متبقي للمبيدات الحيوية المختبرة حيث زاد الأثر الباقي من ٤٩,٩، ٣٠,١٥، ٢٤,٣٤% إلى ٩٥,٠٥ ، ٨٥,١٣، ٧٧,٢٥% على التوالي ضد يرقات العمر الثاني، ومن ٣٠,١٥، ١٢,٧٧، ١٢,٥% إلى ٧٩,٧٥، ٤٩,١٥، ٤٠,٤% ضد يرقات العمر الرابع بعد ١٤ يوم من الرش. هذا بالإضافة إلى أن جميع اليرقات المعاملة تأثرت بيولوجياً بواسطة المبيدات الحيوية المختبرة سواء بمفردها أو مخلوطة مع الزيوت النباتية الثلاثة. اختلف التأثير طبقاً للعمر اليرقي والمبيد الحيوي المختبر والزيت المضاف. وبناء عليه أدت المعاملات إلى خفض في نسب التعذير وخروج الفراشات والوزن العذري والعمر الحشري والخصوبة وفقس البيض وزيادة للبقاء اليرقي والعذري وأحدثت تشوهات يرقية وعذرية وحشرية وأخلت بالنسبة الجنسية للذكور والإناث بالمقارنة بغير المعامل وكانت مخاليط المركبات الحيوية مع زيت السمسم لها التأثير البيولوجي الغالب يليها مخاليط المبيدات مع زيت بذرة القطن بينما احتل زيت فول الصويا الترتيب الأخير.

ويعزي هذا التأثير التنشيطي إلى أن إضافة مثل هذه الزيوت النباتية إلى المركبات الحيوية يؤدي لزيادة كلا من اللزوجة ودرجة الأس الهيدروجيني لمحاليل الرش حيث تؤدي زيادة اللزوجة إلى زيادة التصاق المبيدات على الأوراق النباتية المعاملة وبالتالي زيادة النفاذية وخفض انجراف هذه المبيدات وزيادة استدامة ومقاومة هذه المبيدات للانهيار بفعل أشعة الشمس. أيضاً تؤدي هذه الزيوت إلى خفض معدلات التنفس وزيادة فعالية مبيد الأسبينوساد والذي يعمل على الجهاز العصبي. كما تؤدي هذه الزيوت إلى تغيير في درجة الحموضة في معدة الحشرات لتجعلها أكثر قلوية ومن ثم زيادة فاعلية هذه المركبات البكتيرية كالدابيل ٢ أكس، البروتكتو.