TOXICITY OF SOME INSECTICIDES AND THEIR MIXTURES WITH ERGISTROL BIOSYNTHESIS INHIBITING FUNGICIDES AGAINST THE 2ND INSTAR LARVAE OF *SPODOPTERA LITTORALIS* (BOISD)

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Abstract

The toxicity of organophosphorus insecticide (profenofos), pyrethroid insecticide (esfenvalirate), insect growth regulators, (flufenoxuron) and biocide as natural products (spinosad) increased when mixed with the butylated hydroxyanisol (BHA) and ergosterol biosynthesis inhibiting (EBI) fungicides to 2nd instar larvae of *Spodoptera littoralis*. Present study was carried out by feeding 2nd instar larvae on castor bean leaves treated with different concentrations of profenofos, esfenvalirate, flufenoxuron and spinosad either alone or in mixtures with BHA, EBI fungicide or IGR. Interestingly, these BHA and EBI did not kill the larvae at concentration up to 100 ppm, moreover 20 ppm of each BHA or EBI fungicide with concentrations of the insecticides increased the toxicity of these insecticides. Also, all data indicated that the calculated LC_{50} of the insecticides alone were higher than the insecticides and BHA, EBI fungicides or IGR. For instance, the LC_{50} of profenofos alone was 1.90 ppm, decreased to 0.08, 0.20, 0.24 and 0.22 ppm when mixed with BHA, difenoconazol, penconazol and flufenoxuron respectivily. Also, the insecticidal action increased by 23.75, 9.5, 7.91 and 8.63 folds. The LC_{50} values decreased when esfenvalirate mixed with BHA, difenoconazol, penconazol and flufenoxuron reached 0.32, 0.21, 0.28 and 0.33 ppm compared with 1.58 ppm when esfenvalirate used alone. Also, the insecticidal action increased by 4.93, 7.52, 5.64 and 4.78 folds when mixed with the mentioned BHA, EBI fungicides or the IGR respectively. Concerning flufenoxuron LC_{50} values alone was 1.83 decreased to 0.12, 0.19 and 0.22 ppm when mixed with BHA, difenoconazol, penconazol and flufenoxuron respectively, insecticidal action increased by 15.25, 9.63 and 8.31 folds respectively. LC_{50} values of spinosad was 1.44 ppm when used alone, decreased to 0.21, 0.21, 0.25 and 0.20 ppm when was mixed with BHA, difenoconazol, penconazol and flufenoxuron respectively, insecticidal action was increased by 6.85, 6.85, 5.76 and 7.2 folds respectively. These results suggested that the antioxidant butylated hydroxyanisol (BHA), ergosterol biosynthesis inhibiting (EBI) fungicides and the IGRs enhanced the insecticidal activity of profenofos, esfenvalirate and spinosad against 2nd instar *S.littoralis.*

INTRODUCTION

Cotton growers in Egypt have severe economic losses from cotton pests due to reduced yield, low lint quality and increased costs of insecticides (Amin and Gergis 2006). Insect- pests are considered the important factors that influence cotton production and cause damage to the cotton yield. Cotton leaf worm *Spodoptera littoralis* (Boisd) and the other insect's are the most serious cotton pests in Egypt (Hussein *et al*. 2002). They caused annually the greatest yield losses from the cultivated area. (Haque1991,.El-Naggar1998). Ergosterol biosynthesis inhibiting EBI fungicides is widely used in controlling pathogenic fungus. This group of fungicides inhibits fungi by blocking the cytochrome P-450 dependent demethylation at the C-14 position and thus inhibiting biosynthesis of the principal fungal sterol ergosterol (Ragsdal and Sislar 1991). This group of fungicides has been identified as potent synergists of pyrethroid insecticides in the honey bee. (Colin and Belzunces 1992). For example, 9-16 folds enhanced toxicity was recorded for the pyrethroid insecticides lambadacyalothrin when combined with the EBI fungicides prochloraz and propiconazol respectively, (Pilling and Jepson 1994). The mechanism of this synergism has been studied by (Plling *et al*.1995) reported that prochloraz delayed the metabolism, detoxication, excretion of lambadacyalothrin by inhibiting microsomal oxidation, effectively enhancing the toxicity of pyrethroids to the honey bee. Schmuck *et al*. (2003) found that slight synergistic effect of the thiacloprid to cyprodinil fungicide was observed with honey bee while a significant enhancement was found with EBI fungicides prochloraz and tebuconazol. The insect growth regulator is acting as chitin synthesis inhibitor in insects which confers a remarkable specification with low toxicity to mammals, birds and fish (Flint and Smith 1977). Also, the mixtures between these compounds and traditional insecticides increase the efficiency of these insecticides (Abdel-Sattar and EL-Guindy1988). Also, Raslan (2003) found that all mixtures of 25 ml Spinosad with Consult, Atabron and Dursban at their half- recommended rates per feddan resulted in increasing their activity against the 3rd instars larvae of cotton leaf worm *S. littoralis*. Also, Helalia *et al* (2006) found that the toxicity of the biocides was greatly enhanced when used at low rates of conventional insecticides such as pyrethroids and some organophosphorus compounds in combination with *B. thuringiensis* proved to be suitable to control the cotton leafworm *Spodoptera littoralis*. Abd-EL-Mageed *et al* (2006), reported that spinosad gave moderately initial and residual effect when tested alone whereas the most pronounced initial effect was achieved when

spinosad was mixed with methoxyfenozide. Also, they reported that the highest residual activity was noticed when spinosad was mixed with chlorpyrifos. Arakawa Toru (2008) found that Polyoxin AL a fungicide that inhibits chitin synthesis, showed a synergistic effect with benzoylphenylurea insect growth regulators in killing Spodoptera *litura* larvae. The antioxidant butylated hydroxyanisol which is a food grade chemical, enhanced the activety 0f fungicides thiabendazol and imazalil against *Colletotrichum musae*, the caused fungal pathogen of banana anthracnose (Kan et al., 2001) Additionally Mahmoud and Khalifa (2005) found that BHA increased the herbicide activity of the herbicide bensulfuron-methyl against radish especially with low concentration of the herbicides.

Based on these studies, the present work was conducted to evaluate the synergistic action among the antioxidant butylated hydroxyanisol, ergosterol biosynthesis inhibiting fungicides and the IGRs and both organophosphorus, pyrethroids insecticides and one natural product spinosad against the 2nd instar *S.littoralis*.

MATERIALS AND METHODS

1-INSECTS: -

Laboratory strain obtained as egg-masses from Cotton leaf worm Department, Plant Protection Institute, Agric. Res. Center., Giza. and reared as described by El-Dafrawy *et al.* (1964) under laboratory condition at 25±2˚C and 65±5 % relative humidity. Eggs masses were kept separately in 400 ml glass jar covered with muslin. The jars were provided with castor bean leaves for larval feeding until pupation. The resulting pupae were placed inside each jar, the emerged moths were supplied with a piece of cotton moistened with 10% sugar solution and *Nerium oleander* branches were placed in jars for eggs deposition. The deposited egg-masses were collected daily and were left till hatching. The newly hatched larvae were transferred to fresh castor bean leaves and the instar was differentiated. The newly moulted 2nd instar larvae of *S. littoralis* were used in these studies.

2-CHEMICALs:

The following insecticides, fungicides were employed:-.

1-INSECTICIDES:

1-1- Selecron (profenofos 72 % E.C

1-2 –Sumiat (esvenvalirate 5 %E.C)

1-3-Cascade (flufenoxuron 10 % E.C)

1-4-Spintor (Spinosad 24 % E.C)

2 -**FUNGICIDES**

2- 1- Score (difenoconazol, 25 % E.C)

2 - 2 - Topaz (penconazole, 20 % E. C)

2 -3 -BHA (butylated-hydroxyanisol 100 % w/w)

3-TOXICITY TESTS:

To determine LC_{50} values for each tested compounds used alone or in mixtures with EBI fungicides or insect growth regulators, seven concentrations from each compound were prepared by diluting the formulation product with distilled water 6.5, 3.25, 1.62, 0.81, 0.40, 0.20 and 0.10 ppm for profenofos, and 5, 2.5, 1.25, 0.62, 0.312, 0.156 and 0.078 ppm for esvenvalirate, 5, 2.5, 1.25, 0.62, 0.312, 0.156, and 0.078 ppm for flufenoxuron, and 7.5, 3.75, 1.87, 0.93, 0.46, 0.23, and 0.11 ppm for spinosad. These concentrations were used alone or in mixtures with 20 ppm of EBI fungicides, BHA or 2 ppm of insect growth regulators. Castor bean leaves were dipped for 15 second in each concentration, then left to dry at room temperature and offered to the newly moulted 2nd instar larvae of *S. littoralis*. Three replicates were carried out for each concentration, ten larvae for replicate placed inside each jar, three treated leaves of each concentration were transferred to these glasses. Treated larvae were allowed to feed on the treated leaves for 24 hr, in case of both insecticides and their mixtures with EBI fungicides, BHA but in case insect growth regulators, larvae were fed for 72 hr, then in all cases of feeding periods the larvae were transferred to untreated leaves until pupation. On the other hand, three replicates were dipped in distilled water for the same periods as a check treatment.

Corrected mortality percent was obtained using check treatments and Abbott formula (1925). The corrected percentage of mortality of each compound was staticallycalculated according to Finney (1952) to determine the LC_{50}. Synergistic effects were calculated according to Sun and Johnson (1960)) as follows

$$\text{Synergistic ratio} = \frac{LC_{50} \text{ of insecticides only}}{L\ C_{50} \text{ of insecticides in mixtures}}$$

RESULTS AND DISCUSSION

1-Toxicity of chemical, natural insecticides and their mixtures on the second instar larvae of ***Spodoptera littoralis*** **(Boisd**)

Results in Table (1) indicate that mortality percentage increased with increasing the concentration of tested compounds. For profenofos mortality

percentages of the 2nd instar larvae were 20.0, 26.66, 36.66, 40.0, 43.33, 46.66 and 73.33 % at 0.10, 20, 0.40, 81, 1.62, 3.25 and 6.5 ppm, respectively. Mixing different concentrations of profenofos with BHA resulted in mortality percentages 46.66, 73.33, 83.33, 86.66, 90.0, 96.66 and 100% at the same tested concentrations of profenofos mixed with BHA, respectively. Profenfos mixed with flufenxuron, difenoconazol and penconazol revealed mortality percentages' ranged between 44.82-100, 46.66-100,43.33-100% for profenofos mixtures with flufenxuron, difenoconazol and penconazol respectively. Regarding S-fenvalirate, at 5 ppm , mortality was 75.8 %, while it was 100% after mixing S-fenvalirate with compounds. At 1.25 and 2.5 ppm of S-fenvalirate, mortality percentage of 2nd instar larvae of *S. littoralis* ranged between79.31 and 96.55 % in the different mixtures of S-fenvalirate compared to 44.82 and 48.27% at concentration 1.25 and 2.5 ppm a S-fenvalirate As for flufenoxuron , at 1.25 and 2.5 ppm it gave mortality less than 50 %. That is normal case with IGR compounds, where they revealed low toxicity but they cause highly disruption in the insect biology. Thus, mixing with BHA, difenoconazol, and penconazol revealed synergistic action of flufenoxuron. Cumulative mortality % of flufenoxuron + BHA ranged from 40 to 100% while it ranged from 26.66 to 100 % and 24.13-100 % for flufenoxuron + difenoconazol and flufenoxuron + penconazol, respectively. Taking into account spinosad, data in Table (1) indicate that spinosad concentration up to 3.73 ppm represented mortality % less than 50 %, while revealed 73.33% at 7.5 ppm. Concerning spinosad, BHA, EBI fungicide and IGR mixtures shown in the same table clearly indicate that the spinosad toxicity to larvae was increased by adding the BHA, EBI fungicide or IGR to spinosad concentrations. For instance 0.11 ppm of Spinosad caused 13.33 % larval mortality, but the same concentration when mixed with BHA, EBI fungicides, difenoconazol, penconazol or IGR flufenoxuron, caused 36.66, 26.66, 26.66 and 36.66 % larval mortality respectively. Indeed, spinosad alone at 1.87 caused 40.0 % larval mortality but caused 83.33, 86.66, 90.0, and 86.66% larval mortality when this concentration was mixed with BHA, EBI fungicides, difenoconazol, and penconazol or IGR flufenoxuron. Also, spinosad at 3.75 ppm caused 46.66 % larval mortality when was used alone, but caused 93.33, 90.0, 96.66 and 93.33 % larval mortality when mixed with BHA as antioxidant, EBI fungicides and flufenoxuron as IGR respectively. Interestingly, the spinosad alone at 7.5 ppm induced 73.33 % larval mortality, this concentration caused 100 % larval mortality when was mixed with BHA as antioxidant, EBI fungicides and flufenoxuron as IGR respectively.

2- Synergistic effect of Ergistrol Biosynshesis inhibiting Fungicides on chemical and natural insecticides on the 2nd instar larvae of *Spodoptera littoralis* (Boisd)

Results in Table (2) show the, toxicity and synergistic action of the tested compound used alone or in mixtures with EBI fungicides BHA or IGR at different concentrations to the 2nd instars larvae of *Spodoptera littoralis*. The present data show that LC_{50} values of profenofos, esvenvalirate, spinosad and flufenoxuron were 1.90, 1.58, 1.44 and 1.83 ppm when used alone respectively. Were as the LC_{50} values, were decreased when these compounds were mixed with EBI fungicides BHA or IGR. The LC_{50} values were 0.08, 0.20, 0.24 , and 0.22 ppm when profenofos was combined with BHA, difenoconazol, peconazole as EBI and flufenoxuron respectively. These indicate that insecticidal action increased by 23.75, 9.5, 7.91, and 8.63 folds respectively. Also these values decreased when esvenvalirate was mixed with BHA, EBI, and IGR. These values were 0.32, 0.21, 0.28 and 0.33 ppm respectively, compared with 1.58 ppm when esvenvalirate was used alone. The insecticidal action increased by 4.93, 7.52, 5.64 and 4.78 folds respectively. In addition data indicated that the LC_{50} values were decreased when the spinosad was mixed with BHA, EBI or IGR while these values were 0.21, 0.21, 0.25, and 0.20 ppm. When spinosad mixed with BHA, difenoconazol, peconazol, flufenoxuron respectively, compared with 1.44 ppm for spinosad alone. The insecticidal action increased by 6.85, 6.85, 5.76, and 7.2 folds respectively. These values were decreased when flufenoxuron was combined with BHA, EBI, however these values were 0.12 , 0.19 and 0.22 ppm when mixed with BHA, difenoconazol, penconazol, respectively, compared with 1.83 ppm when this compound was used alone. The toxicity of this compound increased by 15.25, 9.63 and 8.31 folds respectively. Mixtures of butylated-hydroxyanisol, difenoconazol, peconazol or flufenoxuron with different concentrations of organophosphorus insecticides (profenofos) or pyrethroids insecticides (esvenvalirate) or (spinosad) enhanced the toxicity of these insecticides. Interestingly, the BH as antioxidant, fungicides at different concentrations did not cause larval mortality

These results were also reported by (Schmuck *et al.,* 2003) and (Pilling *et al.*, 1995). They found that the EBI fungicides did not have toxic effect to honey bee. It is known that these fungicides inhibit cytochrom P450 monooxygenase system which prevents the biosynthesis ergosterol in fungi. Since, monooxygenase are also involved in the metabolism of organophosphorus, and pyrethroid insecticides (Littele *et al.*, 1989 and Johnston *et al* 1989).Thus fungicides may delay the

toxification, of insecticides by inhibiting monooxygenase system in the insects, and enhanced their toxic action against larvae. Moreover, it has been reported that fungicides that did not inhibit the biosynthesis of ergosterol in fungi did not enhance the toxicity of thiacloprid to honey bee (Schmuck *et al*.,2003). These results also, agree with the previous studies (Colin and Belzunnces 1992). They reported that the fungicides have been identified as synergists of pyrethroids insecticides in the honey bee. (Plling and Jepson 1994). Found that the toxicity of pyrethroid were enhanced from 9-16 folds when combined with EBI fungicides Prochloraz and Propiconazol to honey bee. The insect growth regulators ar acting as chitin synthesis inhibitor in insects which confers a remarkable specification with low toxicity to mammals, birds and fish (Flint and Smith 1977). Also, the mixtures between these compounds (IGRs) and traditional insecticides increase the efficiency of these insecticides (Abdel-Sattar, 1988). Also, (Raslan 2003) found that all mixtures of 25 ml Spinosad with Consult, Atabron and Dursban at their half- recommended rates per feddan resulted in increasing their activity against the 3rd instars larvae of cotton leaf worm. Also, (Helalia *et al*. 2006) found that the toxicity of the biocides was greatly enhanced when using low rates of conventional insecticides such as pyrethroids and some organophosphorus compounds in combination with *B. thuringiensis* proved to be suitable to control the cotton leaf worm *S. littoralis* Abd-EL-Mageed *et al* (2006) reported that spinosad gave moderately initial and residual effect when tested alone whereas the most pronounced initial effect was achieved when spinosad was mixed with methoxyfenozide. Also, they reported that the highest residual activity was noticed when spinosad was mixed with chlorpyrifos. Arakawa (2008) found that Polyoxin AL a fungicide that inhibits chitin synthesis, showed a synergistic effect with benzoylphenylurea insect growth regulators in killing *Spodoptera litura* larvae. The antioxidant butylated hydroxyanisol (BHA) which is a food grade chemical, enhanced the activety 0f fungicides thiabendazol and imazalil against *Colletotrichum musae*, the caused fungal pathogen of banana anthracnose (Kan et al., 2001) Additionally Mahmoud and Khalifa (2005) found that BHA increased the herbicide activity of the herbicide bensulfuron-methyl against radish especially with low concentration of the herbicides.

As mentioned before it could be used as mixtures of these BHA, EBI fungicides or IGR with the insecticides to in increese insecticidal activity at low concentrations.

Table 1. Toxicity of chemical, natural insecticides and their mixtures on the second instar larvae of *S.littralis* .

Treatments	% corrected larval mortality						
Profenofos							
Concentration ppm	0.10	0.20	0.40	0.81	1.62	3.25	6.5
Profenophos	20.0	26.66	36.66	40.0	43.33	46.66	73.33
Profenophos +BHA	46.66	73.33	83.33	86.66	90.0	96.66	100.0
Profenophos+flufenxuron	44.82	66.66	73.33	86.66	93.33	96.66	100.0
Profenophos+difenoconazol	46.66	66.66	70.0	86.66	90.0	96.66	100.0
Profenophos+penconazole	43.33	66.66	73.33	83.33	90.0	96.66	100.0
ES-fenvalirate							
Concentration ppm	0.078	0.156	0.312	0.62	1.25	2.5	5
ES-fenvalirate	24.13	27.58	37.93	41.37	44.82	48.27	75.8
ES-fenvalirate+BHA	28.7	41.37	58.62	68.96	79.31	96.55	100.0
ES-fenvalirate+flufenxuron	31.03	41.37	55.17	68.96	82.75	89.65	100.0
ES-fenvalirate+difenoconazol	36.66	46.66	56.66	86.66	90.0	93.3	100.0
ES-fenvalirate+penconazole	27.58	41.37	62.06	79.31	93.10	96.55	100.0
Flufenoxuron							
Concentration ppm	0.078	0.156	0.312	0.62	1.25	2.5	5
Flufenoxuron	13.33	20.0	23.33	33.33	36.66	46.66	76.66
Flufenoxuron+BHA	40.0	46.66	73.33	83.33	90.0	93.33	100.0
Flufenoxuron+difenoconazol	26.66	46.66	63.33	73.33	83.33	93.33	100.0
Flufenoxuron+ penconazole	24.13	41.37	62.06	72.41	86.20	93.10	100.0
Spinosad							
Concentration ppm	0.11	0.23	0.46	0.93	1.87	3.75	7.5
Spinosad	13.33	20.0	26.66	33.33	40.0	46.66	73.3
Spinosad+BHA	36.66	46.66	63.33	76.66	83.33	93.33	100.0
Spinosad+difenoconazol	26.66	56.66	66.66	70.0	86.66	90.0	100.0
Spinosad+Penconazol	26.66	43.33	56.66	83.33	90.0	96.66	100.0
Spinosad+flufenoxuron	36.66	46.66	66.66	76.66	86.66	93.33	100.0

BHA= butylated hydroxyl anisol

EBI=Ergistrol Biosynthesis Inhibiting fungicides

Table 2. Synergistic effect of Ergistrol Biosynthesis inhibiting fungicides on chemical and natural insecticides on the 2nd instars larvae of *Spodoptera littoralis*

Treatments	LC50	Slope	Synergistic ratio
Profenofos			
Profenofos	1.90	0.65	-
Profenofos +BHA	0.08	1.13	23.75
Profenofos +difenoconazol	0.20	1.16	9.5
Profenofos +penconazol	0.24	1.23	7.91
Profenofos +flufenoxuron	0.22	1.25	8.63
ES-fenvalirate			
ES-fenvalirate	1.58	0.61	-
ES-fenvalirate +BHA	0.32	1.26	4.93
ES-fenvalirate +difenoconazol	0.21	1.16	7.52
ES-fenvalirate +penconazol	0.28	1.66	5.64
ES-fenvalirate +flufenoxuron	0.33	1.16	4.78
Spinosad			
Spinosad	1.44	0.77	-
Spinosad+BHA	0.21	1.18	6.85
Spinosad+ difenoconazol	0.21	1.18	6.85
Spinosad + penconazol	0.25	1.64	5.76
Spinosad +flufenoxuron	0.20	1.23	7.2
Flufenoxuron			
Flufenoxuron	1.83	0.88	-
Flufenoxuron +BHA	0.12	1.05	15.25
Flufenoxuron +difenoconazol	0.19	1.32	9.63
Flufenoxuron +penconazol	0.22	1.39	8.31

BHA= butylated hydroxyl anisol

EBI=Ergistrol Biosynthesis Inhibiting fungicides

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السمية والفعل التنشيطي بين بيوتيلايتيد هيدروكسى انيسول والمبيدات الفطرية المثبطة لتخليق الايرجسترول في الفطريات و بعض المبيدات الحشرية ضد يرقات العمر الثانى لدودة ورق القطن

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معهد بحوث وقاية النباتات– مركزالبحوث الزراعية – الدقي

تمت هذة الدراسة تحت الظروف المعملية بتغذية يرقات العمر الثانى لدودة ورق القطن على ورق خروع معامل بتركيزات مختلفة لكلا من مبيد البرفينوفوس ومبيد اس–فنفليرات ومركب الفيلوفينوكسيرون وكذلك مركب الاسبينوساد حيث استعملت هذة المركبات إما منفردة أو في مخاليط مع بيوتيلايتيد هيدروكسى انيسول **و**بعض المبيدات الفطرية المثبطة لتخليق الايرجسترول في الفطريات وتم تحديد قيم آل LC_{50} لهذة المركبات منفردة أو في مخا لبط لمعرفة مدي زيادة فاعليتها علي الحشرة موضع الدراسة . ودلت النتائج علي أن قيم أل LC_{50}لهذة المبيدات منفردة كانت عالية عنها في حالة استعمالها في مخاليط ، فمثلا كانت قيمة أل LC_{50} لمبيد البرفينوفوس عندما استعمل منفردا ١,٩٠.جزء في المليون، بينما انخفضت هذة القيمة إلي ٠,٠٨ – ٠,٢٠ – ٠,٢٤ – ٠,٢٢ جزء في المليون عندما تم خلطها مع بيوتيلايتيد هيدروكسى انيسول من مضدات الاكسدة و الدايفينوكونازول و البنكونازول من المبيدات الفطرية المثبطة لتخليق الايرجسترول في الفطريات وكذلك مع مركب الفيلوفينوكسيرون من منظمات النمو الحشرية لي الترتيب ، وهذا يدل علي أن سمية هذا المركب ذادت بمقدار ٢٣,٧٥– ٩,٥ – ٧,٩١– ٨,٦٣ ضعف علي التوالي . كذلك كانت قيمة أل LC_{50} لمركب اس–فنفليرات عندما استخدم بمفردة هي ١,٥٨جزء في المليون انخفضت هذة القيمة إلي ٠,٣٢–٠,٢١– ٠,٢٨– ٠,٣٣ جزء في المليون عندما تم خلطها مع بيوتيلايتيد هيدروكسى انيسول و الدايفينوكونازول و البنكونازول وكذلك مع مركب الفيلوفينوكسيرون على الترتيب ، وهذا يدل علي أن سمية هذا المركب ذادت بمقدار ٤,٩٣ – ٧,٥٢ – ٥,٩٤– ٤,٧٨ ضعف علي التوالي بينما كانت قيمة LC_{50} لمركب الفيلوفينوكسيرون عندما استخدم منفردا هي ١,٨٣جزء في المليون **بينما** انخفضت هذة القيمة إلي ٠,١٢– ٠,١٩ – ٠,٢٢ جزء في المليون عندما تم خلطها مع بيوتيلايتيد هيدروكسى انيسول و الدايفينوكونازول و البنكونازول علي الترتيب ، وهذا يدل علي أن سمية هذا المركب ذادت بمقدار ١٥,٢٥– ٩,٦٣– ٨,٣١ ضعف علي التوالي بينما كانت قيمة أل LC_{50} لمركب الاسبينوساد هي ١,٤٤جزء في المليون عندما استخدم بمفردة انخفضت هذة القيمة إلي ٠,٢١– ٠,٢١– ٠,٢٥– ٠,٢٠ جزء في المليون عندما تم خلط هذا لمركب مع بيوتيلايتيد هيدروكسى انيسول و الدايفينوكونازول و البنكونازول وكذلك مركب الفيلوفينوكسيرون من منظمات النمو الحشرية علي الترتيب وهذا يدل علي أن سمية هذا المركب ذادت بمقدار ٦,٨٥ – ٦,٥٨– ٥,٧٦– ٧,٢ضعف علي التوالي علما بأن كلا من بيوتيلايتيد هيدروكسى انيسول و الدايفينوكونازول و البنكونازول لم تسبب موتا ليرقات العمر الثانى من دودة ورق القطن حتى ١٠٠ جزء فى المليون وعلي هذا نقترح استخدام مخاليط هذة المبيدات الحشرية مع المبيدات الفطرية المثبطة لتخليق الايرجسترول في الفطريات أو مع منظمات النمو الحشرية لزيادة فاعلية هذة المبيدات علي يرقات العمر الثانى لدودة ورق القطن مما يؤدي إلي تقليل معدل استخدام هذة المبيدات.