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JOINT ACTION OF BIOINSECTICIDES AND IGRS IN BINARY MIXTURES WITH SEVERAL INSECTICIDES AND THEIR ROLE IN DEVELOPMENT OF RESISTANCE IN *SPODOPTERA LITTORALIS* (BOISD.)

H.S.A. Radwan, M.E. Nassar, A.E. El-Sheikh and
Manal A.A.Abd El-Razik
Pesticide Dept., Faculty of Agric.Minufiya Univ., Egypt.

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ABSTRACT : *The efficacy of 8 binary mixtures representing two naturally derived insecticides, spinosad and abamectin when combined with each of deltamethrin, furathiocarb,methomyl and profenfos at mixing ratios of 9:1, 4:1, 1:1, 1:4 and 1:9 was studied, by feeding 4th instar larvae of S. littoralis (Boisd.) on treated castor been leaves for 24 hr. Also, the joint action of deltamethrin, chlorpyrifos and methomyl when combined with each of four IGRs, methoxyfenozide, chlorfluazuron, hexaflumuron and pyriproxyfen in binary mixtures at the same mixing ratio was studied.Therefore, the acute toxicity (LC_{50}) of each insecticides seperately and those of binary mixtures was assessed. Based on LC_{50} value of each insecticide seperately or the mixtures and mixing ratios, the co-toxicity coefficient (CTC) of mixture was determined.*

At 24h post treatment neither naturally derived compounds in their mixtures showed synergism to the conventional insecticides except for methomyl at limited mixing ratios. However, chlorpyrifos in its mixtures with tested IGRs showed remarkable synergistic activity regardless mixing ratios, whereas deltamethrin in mixture with only methoxyfenozide and hexaflumuron exhibited synergistic activity at limited mixing ratios. In contrast methomyl recorded clear antagonistic action in all mixtures with IGRs, except with chlorfluazuron at 9:1 mixing ratio.

When the most promising mixtures (showing the highest CTC) was used in selection at level LC_{30} for 5 generations, the data obtained indicate remarkable delay in development of resistance in case of spinosad+methomyl (1:4) and chlorpyrifos+hexaflumuron (9:1), compared with relatively higher rate of resistance development for selection with each component alone.

Using synergism from these promising insecticide mixtures should prove to be an additional tool in the overall resistance management strategy.

Key words: *Joint action, resistance, insecticides, IGRs and cotton leafworm*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is a major pest causing enormous losses to many economically agricultural and horticultural crops in Egypt. As, such they have been subjected to widespread intensive chemical control. Owing to its polyvoltine characterestics and serious overlap of generations, this pest easily developed resistance to various kinds of insecticides.

(Murugesan and Dhingra, 1995, Rashwan *et al.*, 1992; El-Bermawy *et al.* 1992, El-Sebae *et al.* 1993; Allam *et al.* 2000 a,b). However, this phenomenon is more alarming because nowadays there are few available common insecticides that are still effective in controlling this pest in Egypt. Therefore, due to lack of alternatives, the management of pest populations and strategies for slowing the elevation of pesticide resistance are essential and have to depend on optimal use of the existing compounds.

During the last three decades, resistance to pesticides is one of the most severe and recurring problems associated with the use of insecticides. However, the development of insecticides resistance is well documented for several insect pests (Denholm *et al.* 1988). Recently, resistance and cross-resistance problems are increasing and the new products have to meet the rising, standards of environmental and toxicological safety (Ware 2000).

One of the most used techniques to avoid selecting for any particular type of resistance; operational programmes, which may apply alternative classes of insecticides in sequence, rotation or mosaics of compounds acting on different target sites (Ahmad *et al.* 2002). Theoretically, under certain conditions, mixtures can delay the development of resistance more effectively than sequences or rotations (Roush 1993), because if resistance to each compound is independent and initially rare, the associated propability of resistance to both compounds is then extremely rare (Curtis 1985). Likewise, Busvine (1970) indicated that not only the mixing of chemicals offers many possibilities in search for better and more potent uses of toxicants, but also it could theoretically prevent the emergence of resistant strains.

In several studies, synergism between insecticides has been reported (Sun and Johanson, 1960, Mansour *et al.*, 1966, Busvine, 1970). Recently it is known that organophosphates (OPs) synergise pyrethroids against several pests (Ali *et al.*, 1977; Asher *et al.*, 1986; Gunning *et al.*, 1999). This type of synergism is explained by inhibition of esterase activity (Gunning *et al.*, 1999). However, synergism has also been reported between the carbamate propoxur and pyrethroids in a pyrethroid-resistant strain of *Culex quinquefasciatus* (Say) (Corbel *et al.* 2003) as well as between the other carbamates, i.e. carbofuran synergizing chlorpyrifos on *Schizaphis graminum* (Rondani) (Archer *et al.*, 1994) and carbofuran synergizing bifenthrin on *Anopheles gambiae* Giles (Corbel *et al.* 2002).

Thus, the present work aims mainly to study

(1) The joint action of binary mixtures of 2 naturally derived insecticides (spinosad and abamectin) and 4 traditional insecticides (deltamethrin, furathiocarb, methomyl and profenfos).

(2) The joint action of binary mixtures of 3 traditional insecticides (deltamethrin, chlorpyrifos and methomyl) and 4 insect growth regulators (methoxyfenozide, chlorfluazuron, hexaflumuron and pyriproxyfen).

(3) Studying the rate of resistance development in selection study using binary mixtures for 5 generations, compared with selection using each component separately.

MATERIALS AND METHODS

1. Insects

Field strain eggmasses were collected from cotton fields of Behera Governorate West Delta of Egypt at which the cotton leafworm larvae have been exposed to field routine selection pressure of certain conventional insecticides that are usually applied every year from June-September in the governmental official chemical control program. The routinely applied insecticides include organophosphates as chlorpyrifos ethyl and profenfos, pyrethroids as cypermethrin, carbamates as carbaryl and insect growth regulators as chlorfluazuron, hexaflumuron (Temerak 2002).

The cotton leafworm egg masses were collected during June, and after mixing together before hatching, subsample of around 1000 one were reared on castor bean leaves under 27 °C and 55-60 % ± 5 relative humidity according to El-Defrawi *et al.* (1964) technique.

2. Insecticides

Three groups of formulated insecticides were chosen for investigation either alone or in mixtures. These insecticides includes: naturally derived insecticides: spinosad (spintor 24 SC), and abamectin (Vertemic 1.8 % EC); the conventional insecticides: deltamethrin (Decis 2.5% EC, furathiocarb(Deltanet 40% EC), methomyl (Lannate 90% SP), profenfos (Curacron 72% EC) and chlorpyrifos ethyl (Dursban 48% EC), insect growth regulators: methoxyfenozide (Runner 24% SC), chlorfluazuron (Atabron 5% EC), hexaflumuron (Consult 10 % EC) and pyriproxyfen (Admiral 10 % EC).

3. Bioassay

The leaf dipping technique was adopted for the toxicity bioassay of each insecticide tested separately or in binary mixture. Insecticide solutions of a series of concentrations were prepared fresh daily. Castor bean leaves were dipped into these solutions for 20 seconds, air dried at room temperature. Leaves treated with water alone were used as control. For each concentration 10 fourth instar larvae of *S. littoralis* were introduced into glass jar (1L) and were offered either treated or untreated castor bean leaves for 24 h and then replaced by untreated leaves. Castor bean leaves were renewed daily for 72 h. Fresh stock solutions of formulated sample of each insecticide alone or in mixture were prepared daily on the basis of weight per volume of a.i. then serially diluted to the tested concentrations. On the basis of preliminary experiments at least 6 concentrations of insecticides that caused mortality ranged from 20 to 90 % were used to determine their LC_{50} values. In all tests, 30 newly moulted (within 24 h after ecdysis) fourth instar larvae of *S. littoralis* were used for each mixture and mixture component. Concentrations were evently placed around the 50 % response level.

Data mortality was recorded at 24, 48 and 72 h and was subjected to Abbott formula (Abbott 1925) for mortality correction wherever required. Probit analysis was determined to calculate LC_{50} values (Finney, 1977). The co-toxicity coefficient based on the LC_{50} values was calculated according to method of Sun and Johanson (1960), using toxicity index (Sun, 1950).

RESULTS AND DISCUSSION

The joint action data shown in Table (1) illustrate that both naturally derived compounds, spinosad and abamectin in combinations with the four traditional insecticides profenfos, furathiocarb, methomyl and deltamethrin at most of the tested mixing ratios exerted an antagonistic action based on the co-toxicity coefficient calculated on the basis of LC_{50} values at 24 or/and 72 hrs. However, slight synergistic action was recorded when spinosad was combined only with methomyl at mixing ratios of 4:1, 1:4 and 1:9 (spin.+meth.) and when abamectin was also combined with methomyl at mixing ratio of 4:1 (aba+meth).

As for combinations of the traditional insecticides, deltamethrin, chlorpyrifos and methomyl with the four tested insect growth regulators, methoxyfenozide, chlorfluazuron, hexaflumuron and pyriproxyfen at the same testing ratios (Table 2 and 3), it was obvious that the effectiveness of the traditional insecticides in binary mixtures with IGRs varied according to either the tested insecticide or/and the IGR as well as with the mixing ratios of both component in the mixtures. Generally, moderate to high synergism was achieved and resulted in co-toxicity coefficient values ranged between 106.61 to 3248.71 after 24 hr (Table2) and between 105.97 to 37043.83 after 72 hr posttreatment (Table 3)

As shown in Table (2) mixtures of chlorpyrifos with the four IGRs at different mixing ratios exhibited remarkably considerable synergistic action where the co-toxicity coefficient reached 232.88 to 3248.71, 127.25 to 2129.04, 106.61 to 398.80 and 483.55 to 807.05 for its mixtures with methoxyfenozide, chlorfluazuron, hexaflumuron and pyriproxyfen, respectively. On contrary, methomyl/IGR combinations evoked mostly highly pronounced antagonistic action at 24 h posttreatment, except for methomyl+chlorfluazuron (9:1) where moderate synergistic action (Cotox coeff = 123.52) was achieved.

Regarding the degree of synergism (Table 2), it was evident that deltamethrin revealed its highest synergistic action when combined with hexaflumuron at ratios of 1:4 and 1:9 (delt+hexa) and also when combined with methoxyfenozide at ratios of 1:1, 1:4, and 1:9 (delt=meth), resulting in co-toxicity coefficient values of 535.13 and 134.99 for hexaflumuron mixtures and 156.33, 117.80 and 243.99 for methoxyfenozide mixtures at the prementioned mixing ratios, respectively.

Table (1):Joint action and co-toxicity coefficient of 2 naturally derived insecticides in binary mixtures with 4 traditional insecticides against the 4th instar larvae of *S.littoralis* field strain after feeding or/and exposure period of 24 and 72 hr post treatment

Insecticide mixtures		Co-toxicity coefficient values at the indicated mixing ratios after 24 and 72 hr exposure period post treatment.				
		9:1	4:1	1:1	1:4	1:9
				24 hr post treatment		
spinosad	deltamethrin	112.53	23.76	4.26	30.07	19.82
	furathiocarb	4.25	6.47	7.04	0.93	7.61
	methomyl	35.27	106.92	63.59	104.94	112.00
	profenfos	67.80	58.86	54.93	66.05	71.69
abamectin	deltamethrin	13.02	13.25	6.00	12.21	21.14
	furathiocarb	7.88	43.69	7.00	12.58	8.57
	methomyl	27.20	138.86	29.20	40.96	79.46
	profenfos	3.17	2.70	1.89	0.51	3.48
				72 hr post treatment		
spinosad	deltamethrin	7.20	3.62	3.48	3.02	7.79
	furathiocarb	1.12	0.56	0.63	4.72	0.61
	methomyl	2.88	20.25	5.68	9.70	10.04
	profenfos	4.68	6.20	5.93	9.36	18.90
abamectin	deltamethrin	3.01	5.76	11.64	2.67	4.85
	furathiocarb	3.76	4.97	2.12	1.96	2.28
	methomyl	4.52	4.08	1.27	0.64	2.68
	profenfos	8.02	268.81	91.45	2.26	6.32

Table (2): Joint action and co-toxicity coefficient of 3 traditional insecticides in binary mixture with 4 insect growth regulators against 4th instar larvae of cotton leafworm field strain at 24 hr post treatment.

Insecticide mixtures		Co-toxicity coefficient values at the indicated mixing ratios after feeding for 24 on treated leaves				
		9:1	4:1	1:1	1:4	1:9
deltamethrin	methoxyfenozide	75.60	66.59	156.33	117.80	243.99
	chlorfluazuron	44.35	20.40	43.43	2.74	11.31
	hexaflumuron	13.82	20.01	11.77	535.13	134.99
	pyriproxyfen	0.79	3.87	0.18	3.04	3.02
chlorpyrifos	methoxyfenozide	324.33	260.08	3248.71	320.94	232.88
	chlorfluazuron	127.25	180.70	1658.62	2128.90	2129.04
	hexaflumuron	146.04	398.80	113.62	259.38	106.61
	pyriproxyfen	20.22	10.22	60.17	807.05	483.55
methomyl	methoxyfenozide	35.78	19.99	63.34	41.87	22.94
	chlorfluazuron	123.52	76.68	34.34	85.34	75.66
	hexaflumuron	32.05	56.71	41.58	92.11	81.18
	pyriproxyfen	22.61	14.27	12.54	44.14	4.95

Table (3): Joint action and co-toxicity coefficient of 3 traditional insecticides in binary mixture with 4 insect growth regulators against 4th instar larvae of cotton leafworm field strain at 72 hr post treatment

Insecticide mixtures		Co-toxicity coefficient values at the indicated mixing ratios after feeding for 72hr on treated leaves				
		9:1	4:1	1:1	1:4	1:9
deltamethrin	methoxyfenozide	325.12	196.07	495.45	935.30	928.10
	chlorfluazuron	68.92	161.41	148.59	30.44	36.62
	hexaflumuron	154.96	264.43	749.92	658.55	37043.83
	pyriproxyfen	1.73	5.39	3.56	36.91	22.57
chlorpyrifos	methoxyfenozide	337.60	117.70	—	—	18.27
	chlorfluazuron	107.89	119.75	—	519.69	351.86
	hexaflumuron	1215.06	1360.91	189.26	28.60	43.06
	pyriproxyfen	25.10	15.03	72.81	137.87	451.98
methomyl	methoxyfenozide	3872.97	26.79	106.93	112.20	1665.88
	chlorfluazuron	177.19	69.62	84.82	105.97	40.58
	hexaflumuron	97.47	24.80	56.53	39.82	155.72
	pyriproxyfen	18.48	12.92	26.61	88.74	55.79

Considering the response of the 4th instar larvae to different tested mixtures at 72 hr posttreatment (Table 3), it was obvious that almostly moderate increase in performance was achieved, where remarkably higher increase in synergistic action was recorded, particularly for deltamethrin in mixtures with methoxyfenozide, chlorfluazuron and hexaflumuron as manifested by co-toxicity coefficient values of 196.07 to 935.3; 148.59 to 161.41 and 154.96 to 37043.83, respectively.

As for chlorpyrifos/IGRs mixtures, varying degrees of synergisitic action was produced at 72 h (Table 3), reached 117.7 to 337.60; 107.89 to 519.69; 189.26 to 1360.91 and 137.87 to 451.98 when the compound was tested in mixtures with methoxyfenozide, chlorfluazuron, hexaflumuron and pyriproxyfen, respectively. On the other hand mixture of methomyl/IGRs exhibited a highly pronounced increase in synergistic action with only methoxyfenozide recording co-toxicity coefficient of 3872.97 and 1665.88 at mixing ratios of 9:1 and 1:9 whereas methomyl/chlorfluazuron (1:9) and methomyl/hexaflumuron (1:9) resulted in moderately synergistic action expressed by co-toxicity coefficient of 177.19 and 155.72, respectively.

A general model has been developed to explain synergism between insecticides which indicate that one toxicant interferes with the metabolic detoxification of the second toxicant, thereby potentiating the toxicity of the latter compound (Corbett, 1974), whereas antagonism results when interference with the activation mechanisms occurs. On the basis of this general hypothesis an explanation for all the data obtained in this present work can be offered.

In conclusion it is of great interest to observe that the mixtures of the tested IGRs and chlorpyrifos in particular exhibited considerably high potentiation at all mixing ratios. Similar findings were achieved by Radwan *et al.* (1983) where chlorpyrifos/diflubenzuron at 480/40 g.a.i./fed (12:1) manifested *S. littoralis* larval toxicity after 24 hr higher than with the insecticide alone. Likewise, Moustafa and El-Attal (1984) found that equitoxic binary mixtures of chlorpyrifos/triflumuron (EC_{25}+EC_{25}) produced high level of synergism based on percent inhibition of adult emergence. Also, our data are in agreement with Abdallah and Kandil (1985) where they found that chlorpyrifos acts as potentiator for several insecticides belonging to different groups including mainly antimoulting compounds (IGRs). Likewise, Ahmad *et al.* (2008) found the combination indices of cypermethrin + chlorpyrifos at 1:1 and 1:10 ratios and cypermethrin + fipronil at 1:1, 1:10 and 1:20 ratios for the field strain were significantly below 1, suggesting synergistic interactions. and that chlorpyrifos, profenfos and fipronil could be used in mixtures to restore cypermethrin and deltamethrin susceptibility,which may have considerable practical implications for *S. litura* resistance management.

As for deltamethrin, slight to moderate synergistic activity was achieved when combined with methoxyfenozide at mixing ratios of 1:1, 1:4 and 1:9 and also, when combined with chlorfluazuron at mixing ratio of 9:1.

As for methomyl in combinations with IGRs it was obviously demonstrated that all combinations exhibited remarkable antagonism, particularly its combinations with the JHM pyriproxyfen (Table 2 and 3) which is in agreement also with finding of El-Guindy *et al* (1983) recording remarkable antagonism when methomyl was mixed with the JHA triprene. On contrary El-Guindy *et al.* (1983) indicated that diflubenzuron (IGR) produced low level of synergistic action when combined with methomyl against 4th instar larvae of *S. littoralis.*

Data in Table (4) revealed that insecticides resistance developed at variable rates in all cotton leafworm populations under selection during the study. Selection with chlorpyrifos, methoxyfenozide, spinosad, methomyl, deltamethrin, abamectin and hexaflumuron separately resulted in resistance development by tolerance ratio (TR) of 0.60, 1.71, 1.87, 2.22, 3.4, 4.01 and 4.63-fold, respectively in generation F2, which increased under single continuous selection to RR of 5.79, 8.91, 6.8, 6.15,21.25, 9.6 and 8.49-fold, respectively by generation F5. However, spinosad (6.8-fold) when combined with either methomyl (6.15-fold) or deltamethrin (19.6-fold) performed better at F5 and resulted in 4.74 and 10.82-fold for its binary mixtures with methomyl and deltamethrin, respectively, at 24 h post treatment. On the other hand deltamethrin (21.49-fold) when combined with either hexaflumuron (8.49-fold) or methoxyfenozide (8.91-fold) resulted in relatively less development of resistance expressed as RR of 10.78 and 10.26-fold, respectively in F5 at 24 h post treatment. Comparing with single continuous insecticide selections, the onset and degree of resistance development was considerably reduced by mixing insecticides as manifested by spinosad+methomyl (1:4), recording RR = 4.74-fold at F5 compared with 6.8 and 6.15-fold for the single continuous selection by its components, respectively. Similarly, Mckenzie and Byford(1993) came to the same findings where selection with insecticide mixture of permethrin+diazinon (1:2) resulted in the delay of any apparent resistance development in population of horn fly for an additional one to seven generations.

Likewise, Prabhaker *et al.* (1998) found that resistance development in *Bemisia argentifolii* by mixture of bifenthrin+endosulfan (1:2) was developed and recording RR of 17-fold after 23 generations compared with 752-fold for single continuous selection with bifenthrin by generation F27.

Finally application of mixtures exhibiting synergistic action will permit growers to attain the benefits of an antiresistance strategy at reduced cost and insecticide input into the environment. Nevertheless, there is need to strength research on the synergistic effects of other insecticides or and IGRs.

Table (4): Rate of development of resistance at 24 and 72 hr in *S. littoralis* field strain after exposure to selection at LC_{30} level with insecticides alone or/and in binary mixtures for 5 successive generations.

Component of the mixture		Component Ratio	Resistance ratio after exposure of the indicated generation (G) to selection with mixtures at LC_{30} level					
1	2	Comp.: Comp.	Component 1		Component 2		Mixture	
		1 : 2	G2	G5	G2	G5	G2	G5
					24 hr post treatment			
abamectin	methomyl	4 : 1	4.01	9.6	2.22	6.15	2.43	14.36
spinosad	deltamethrin	9 : 1	1.87	6.8	4.01	19.6	2.98	10.82
spinosad	methomyl	1 : 4	1.87	6.8	2.22	6.15	2.50	4.74
chlorpyrifos	hexaflumuron	9 : 1	0.60	5.79	4.36	8.49	3.30	8.82
deltamethrin	hexaflumuron	1 : 9	3.40	21.25	4.63	8.49	2.88	10.78
deltamethrin	methoxyfenozide	1 : 9	3.40	21.25	1.71	8.91	1.96	10.26
					72 hr post treatment			
abamectin	methomyl	4 : 1	3.18	2.20	2.70	2.94	3.5	1.90
spinosad	deltamethrin	9 : 1	3.90	3.70	2.30	1.23	3.72	4.13
spinosad	methomyl	1 : 4	3.90	3.70	2.70	2.94	1.75	1.60
chlorpyrifos	hexaflumuron	9 : 1	0.87	2.50	2.60	2.08	1.0	0.03
deltamethrin	hexaflumuron	1 : 9	1.14	2.58	2.60	2.08	0.03	1.42
deltamethrin	methoxyfenozide	1 : 9	1.14	2.58	1.84	2.43	1042	2.03

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التأثير المشترك للمبيدات الحشرية الحيوية و منظمات النمو الحشرية فى مخلوط ثنائى مع عديد من المبيدات الحشرية التقليدية و دورها فى تطور المقاومة فى دودة ورق القطن

حسنى سيد احمد رضوان ، مصطفى السيد نصار ، انور السيد الشيخ ،
منال عبد الرءوف عبد المجيدعبد الرازق
قسم مبيدات الافات – كلية الزراعة –شبين الكوم–جامعة المنوفية

الملخص العربي

تم دراسة التأثير المشترك لخلط مركبين من المبيدات الطبيعية : اسبينوساد ، ابامكتين مع كل من الدلتامثرين , الفيوراثيوكارب ، الميثوميل و البروفينفوس عند نسب خلط ٩:١ ، ٤:١ ، ١:١ ، ١:٤ ، ١:٩ و تغذية يرقات العمر الرابع من دودة ورق القطن عليها لمدة ٢٤ساعة .

كذلك تم دراسة الفعل المشترك لخلط مبيدات دلتامثرين ، كلوربيروفوس ، ميثوميل مع ٤ من منظمات النمو الحشرية و هى ميثوكسى فينوزيد ، كلورفلوازيرون ، هكسافلوميرون ، البيروبروكسيفين بنفس النسب السابقة وتم تقدير قيمة ال LC_{50} لكل من المخلوط و كلا مكوناتة منفردة و منها تم حساب معامل التنشيط **Co toxicity coefficient**

وقد دلت النتائج على انة بعد ٢٤ ساعة من المعاملة لم يظهر ايا من المبيدات الطبيعية تاثير منشط مع المبيدات الحشرية التقليدية ماعدا الميثوميل الذى اظهر تاثير منشط طفيف عند نسب خلط ٤:١ , ١:٤ و ٩:١ مع الاسبينوساد و ٤:١ مع الابامكتين.

اما بالنسبة للمبيدات التقليدية فقد اظهر الكلوربيرفوس تاثير منشط ملحوظ مع منظمات النمو الحشرية المختبرة عند الغالبية العظمى من نسب الخلط , بينما اظهر الدلتامثرين تاثير منشط مع الميثوكسي فينوزيد و الهكسافلوميرون فقط عند نسب خلط محدودة . على العكس اظهر الميثوميل تاثير مضاد ملحوظ مع كل نسب خلطة مع منظمات النمو الحشرية.

كذلك اوضحت نتائج الانتخاب لمدة ٥ اجيال متتالية ل ٦ من المخاليط ذات نتائج التنشيط المشجعة وجود تاخير ملحوظ فى تطور المقاومة فى حالة مخاليط اسبينوساد + ميثوميل (٤:١) و مخلوط كلوربيروفوس + هكسافلوميرون (١:٩) مقارنة بصورة اسرع نسبيا لكل من مكونات المخلوط منفردة لمدة ٥ اجيال ايضا.

استخدام التنشيط فى المخاليط الواعدة من هذة المبيدات الحشرية يقدم اداة اضافية فى استراتيجية ادارة المقاومة.