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THE STATUS OF PYRETHROID RESISTANCE IN FIELD-COLLECTED STRAINS OF THE *Spodoptera littoralis* AND ROLE OF PIPERONYL BUTOXIDE.

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ABSTRACT

Piperonyl butoxide, or PBO as it is most often called, is a pesticide synergist. A synergist is another chemical that is added to a pesticide product, in addition to the active and inert ingredients, to increase the potency of the active ingredient. Development of insecticide resistance has been a challenging problem for unlimited time and new solutions are yet to emerge. The use of synergist with the insecticide is thought to play a key role in reducing the resistance levels. The availability of a synergist was important because there were limited supplies of Pyrethroid available. Present study demonstrates the efficacy of PBO with pyrethroid insecticides available for controlling *Spodoptera sp.* Generally the 4th instar larvae from 4 agriculture region were collected; bioassay technique and toxicity lines were achieved. Data obtaind showed that PBO were mostly effective to synergy any of those insecticides tested in all regions except for esfenvalerate and α -cypermethrin in Fayoum and fenvalerate in Sharkia. Evenly all insecticide were 99% suppressed in resistance levels except for α -cypermethrin in Fayoum was 80%. This suggests that when Organophosphorus appears to be not effective rapidly and the pest becomes highly resistance the use of pyrethroids always then needed. Subsequently when the pest developed resistance towards pyrethroids then piperonyle butoxide may have stop pyrethroid resistance when it happened. But when piperonyl butoxide becomes ineffective, we can try to use OP's compounds again.

INTRODUCTION

Due to differences in temperature and cropping patterns, such as vegetables, crops and cotton or other hay there is a wide range of *Spodoptera litura* or *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), infestation specially cotton plant in Egypt (Abul-Nasr *et al.*, 1968, Mochida 1973). The high damage to plant foliage required a great variation in insecticides spray that track widespread occurrence of resistance to insecticides is a serious threat for the control and management of *S. littoralis* (Abo-Elghar *et al.*, 2005). The use of Pyrethrins and their synthetic analogues, (Pyrethroid insecticides) that produce rapid killing to insects by generating a very rapid paralysis (knockdown), high opportunities to preserve its efficacy to continue is in attendance. Ever since of their non-persistence in field conditions, pyrithrins have limited use in agriculture pest control. Lots of trials to investigate the efficacy problems of many varieties of pyrethroids were performed around the world such as monitoring of resistance and detoxifications by enzymes or Pyrethroid synergism by esterase inhibition and Efficacy of insecticide mixtures against pyrethroid (Ishaaya 1983 and 1993). Synergists have been used commercially for about 50 years and have contributed significantly to improve the efficacy of insecticides, particularly

when problems of resistance have arisen. These natural or synthetic chemicals, which increase the lethality and effectiveness of currently available insecticides, are by themselves considered nontoxic. The mode of action of the majority of synergists is to block the metabolic systems that would otherwise break down insecticide molecules. A mostly they interfere with the detoxication of insecticides through their action on poly substrate monooxygenases and other enzyme systems (Casida, 1970). The good Synergists are that inhibit the enzymes that catalyze this metabolic degradation and thereby enhance the insecticidal activity.

To monitor resistance seasonally, grower should define a dose that is indicative of particular acceptable resistance ratio and use this discriminating dose to discriminate between resistant and susceptible of individual larvae for pest monitoring and predict whether economic control will be achieved with the pesticide tested (Abo-Elghar *et al.,* 2005). This will not only establish a good data base for region wide monitoring, but will also aid in quick diagnosis of any shifts in resistance and to decide whether failures of chemicals were due to the development of resistance or due to faulty application methods or timing (Heinrichs *et al.,* 1982). Roach and Miller (1986) mentioned that when R gene frequencies >10%, resistance is already established in the population. The contribution between the chemical synergist and the discriminating concentration assay were carried out to provide an alternation of compounds from different chemical classes and synergy the existent insecticide and leftovers an entirely viable resistance management technique by define a set of frequencies of resistance that measured a practice that minimize selection pressures and would predict control failure at these pest densities.

MATERIALS AND METHODS

Insects:

S.littoralis populations were collected from Behera, Sharkia, Dakahlia and Faypum cotton and vegetable crops such as cabbage and cauliflower for carrying out the all insecticide bioassay testing. Laboratory reference strain were reared for more than ten years on leaves of castor bean *Ricinus communis* and kept away from insecticide exposure in the laboratory at 25 ± 2°C and 60:65% RH with a photoperiod of 14:10 (L:D) h. Fresh leaves were replaced after 24 h, and pupae were collected on alternate days. The emerged adults were kept in woody oviposition cages with meshed sides to maintain ventilation. They were fed on a solution containing sucrose (100 g/liter). The adults were allowed to oviposit on fresh leaves of *Nerium oleander.* Eggs patches put inside containers immediately subsequent to ovipositon terminate.

Bioassays:

Bioassay dipping methods were employed in the toxicity tests on the newly molted fourth instar (3–6 h old) larvae of *S.littoralis* for both field and laboratory cultures. Tested subsequent solutions of formulated insecticides were instantly diluted in water. For testing the synergistic effect of PBO, stock

solutions of the tested insecticides and PBO (PBO, 90.0%) were mixed at 1:4 (vol:vol) ratio. To avoid the mortality by synergists, the concentrations used were 30 ppm of PBO which when applied alone without the insecticides resulted in no mortality. The feeding supplies was cotton leaves collected from unsprayed fields were washed, dried and immersed in a test solution for 10 s and allowed to dry at for one hour subsequently placed in individual Petri dishes (10-cm diameter). Inside each dish ten larvae were placed on each leaf. For control treatment cotton Leaf immersed in distilled water only. Each treatment (concentration) was replicated three times, including controls. The bioassays were kept at a temperature of 25± 1C, 65% relative humidity and 14:10 (light: dark) photoperiod. Mortality was assessed after 24 h exposure to insecticides. Larvae were considered dead if they gave no coordinated response to stimulation by touch with a blunt needle.

Statistical analysis:

Results were expressed as percentage of mortality, correcting for untreated (control) mortality using Abbott's (1925) formula. Data were analyzed Polo Pc Program analysis (Rusell *et al.*, 1977). Resistance ratios were determined by dividing the LC_{50} values of field populations by LC_{50} of Laboratory strain. The synergistic ratio is calculated by dividing the LC_{50} value of tested insecticide by the LC_{50} value of tested insecticide plus PBO. The percent suppression in tested insecticide resistances by PBO was computed as described by Fakoorziba *et al.*, (2009), which is as follows:

$$\%\,\text{suppression in resistance} = 1 - \left[\frac{LC_{50}\text{ of each insecticide with PBO}}{LC_{50}\text{ of each insecticide alone}}\right] x100$$

The estimated heterogeneity (H) values with its probability in probit analysis were used to find out whether the population is homogenous (P [0.05]) or heterogeneous (P \0.05) for resistance to the insecticides concerned (Hoskins and Craig 1962). The mortality data were subjected to regression analysis of probit-mortality on log dosage, their LC_{50}, slope, and heterogeneity about the linear regression line was computed according to (Finney, 1971). Strains were considered significantly different if their 95% confidential limits of the LC_{50} did not overlap.

Gene frequency estimates were done as described by Cochran (1994a and 1994b). They were based on the Hardy-Weinberg equilibrium expression (Falconer, 1981). The discriminative concentration means the concentration that kill 99% of the susceptible proportion for each insecticide which recorded gene frequency (GF). Gene frequency equals the square root of the fraction representing the survivors in a test sample from a field population. The calculation depend on all homozygous susceptible and heterozygous individuals are killed by the pesticides in the test protocol used (Ebbett and Cochran, 1997); Thus, only homozygous resistant individuals survive.

RESULTS AND DISCUSSION

Results of the leaf-dip assays of the susceptible strain are summarized in Table 1 and the four field strains in Tables 2, 3, 4 and 5. LC_{50} estimates of susceptibility to all insecticides tested of field Behera, Dakahlia, Sharkia and Fayoum populations were significantly different from the LC_{50} of the susceptible strain. Resistance ratio (RR) of all insectcicides tested in of all regions was ranged from 2.4 to 1972.9 (Table 2, 3, 4, and 5). But when Insecticide resistance levels was classified using RRs in terms widely accepted as follows: susceptibility (RR =1), tolerance to low resistance (RR =2–10), moderate resistance (RR= 11–30), high resistance (RR =31–100) and very high resistance (RR>100) (Ahmad *et al.*, 2008), then all regions were highly resistance to all insecticides according to the previous discribtions of RR. Behera region were higher resistance to all insecticides at all in comparison with Dakahlia, Sharkia and Fayoum.

Table 1: The response of the susceptible *S.littoralis* strain.

Pesticide name	Slope±SE	LC_{50} (Limits)	LC_{90} (Limits)	H	G	DC
Lambdacyhalothrin	1.539+-0.227	0.072 (0.051 - 0.099)	0.493 (0.310 - 1.065)	0.33	0.083	2.354
Cypermethrin	1.938+-0.257	0.091 (0.069 - 0.118)	0.418 (0.291 - 0.731)	0.61	0.067	1.448
α-cypermethrin	1.668+-.230	0.425 (0.316 - 0.572)	2.489 (1.577 - 5.201)	0.68	0.073	10.527
Delta-methrin	1.426+-.217	0.083 (0.057 - 0.115)	0.655 (0.389 - 1.590)	0.05	0.089	3.539
Permethrin	1.565+-0.226	0.106 (0.077 - 0.144)	0.702 (0.439 - 1.520)	0.21	0.08	3.264
Fenvalerate	1.476+-0.219	0.173 (0.122 - 0.239)	1.279 (0.771 - 2.993)	0.54	0.085	6.524
Es-fenvalerate	1.539+-.222	0.093 (0.067 - 0.127)	0.633 (0.390 - 1.412)	0.24	0.08	3.023
Fenpropathrin	1.640+-.230	0.374 (0.275 - 0.503)	2.261 (1.438 - 4.704)	0.58	0.075	9.799

H: heterogeneity factor is equal to χ^2 divided by d.f.,
G: G-test, it is also called a log-likelihood test or a likelihood ratio test = G = t2 x Sb2 / b2
Dc: Discriminative concentration =

The synergistic ratio (SR) of all insecticides are in tables 2, 3, 4 and 5 provide a sight about how much the synergist effect; suggest itself and what insecticide were responded to the synergist applied. All insecticides tested in all regions responded to PBO then there isn't Multiplicity of resistance mechanisms. Another observation is the difference in the levels of activity to each insecticide in the presence of PBO and without it was much higher; this indicates that there isn't several independent metabolic resistance mechanisms were present in those strain. This is an evidence to the PBO were mostly effective to synergy any of those insecticides in all region, but PBO was ineffective against fenvalerate in Sharkia, es-fenvalerate and α - cypermethrin in Fayoum this mean that PBO failed to block resistance.

Esfenvalerate and α-Cypermethrin in Fayoum and fenvalerate in Sharkia mixed with Piperonyl butoxide have very little synergistic ratio. Many searches may interprete the condition. There is a doubt that multible resistance are exist in the population but synergy with PBO because there is an improvement of the insecticide penetration into cuticle. Gunning *et al.*, 1999 found that the reduced penetration of esfenvalerate in the resistant *Helicoverpa armigera* larvae appeared to be an important resistance mechanism.

Table 2: Toxicity of the insecticides with PBO to Behera 4th instar *S.littoralis*.

Pesticide name	LC_{50} (Limits)	Slope±SE	H	RR	M	Gf	LC_{50}+ PBO	Slope±SE	H	SR	%S
Lambdacyhalothrin	48.51 (30.6 - 66.9)	1.645±0.296	0.1	673.7	1.6	0.992	0.93 (0.53 - 1.52)	0.847±0.157	0.08	52.16	98.08
Cypermethrin	179.54 (130.8- 236.7)	1.89±0.299	0.11	1972.9	26.51	0.8572	2.76 (2.0 - 3.7)	ı.7±0.286	0.86	65.05	98.46
α -cypermethrin	31.36 (22.4 - 41.4)	1.899±0.30	0.05	73.8	18.42	0.9032	0.125 (0.053 - 0.20)	1.06±0.215	0.09	250.88	99.60
Delta-methrin	33.83 (21.3-46.2)	1.79±0.389	0.12	407.59	3.93	0.9801	0.345 (0.20 - 0.5)	1.36±0.228	0.28	98.06	98.98
Permethrin	5.78 (2.7 - 10.3)	1.397±0.219	1.39	54.53	35.53	0.8029	0.28 (0.16 - 0.40)	1.32±0.27	0.14	20.64	99.52
Fenvalerate	207.64 (116.8 - 303)	1.33±0.276	0.54	1200.2	2.312	0.9884	3.35 (2.2 - 4.6)	1.5±0.23	0.19	61.98	98.39
Es-fenvalerate	80.37 (47.6 - 111)	1.76±0.397	0.73	864.16	0.7	0.9965	0.664 (0.455 - 0.93)	1.38±0.216	0.57	121.03	99.17
Fenpropathrin	240.28 (78.9 - 442.7)	1.51±0.283	1.17	642.46	1.84	0.9907	4.725 (2.8 - 7.0)	1.11±0.20	0.08	50.85	98.03

SR: synergism ratio
%S: percentage of resistance suppression by synergism
RR: resistance ratio LC_{50} of the field stain divided by the LC_{50} of the susceptible
GF: gene frequency

Table 3: Toxicity of the insecticides with PBO to Dakahlia 4th instar *S.littoralis*.

Pesticide name	LC_{50} (Limits)	Slope±SE	H	RR	M	Gf	LC_{50}+ PBO	Slope±SE	H	SR	(%) S
Lambdacyhalothrin	6.86 (4.3 - 9.6)	1.53±0.284	0.54	95.3	23.92	0.872	0.154 (0.10 - 0.22)	1.31±0.21	0.3	44.55	97.76
Cypermethrin	85.54 (44.1 - 121.6)	1.68±0.40	0.43	940	0.15	0.999	1.71 (1.12 - 2.34)	1.64±0.294	0.57	50.02	98.00
α -cypermethrin	34.99 (17.0 - 61.6)	1.47±0.224	1.43	82.33	22.08	0.883	0.277 (0.21 - 0.36)	1.92±0.257	0.58	126.32	99.21
Delta-methrin	6.158 (4.3 - 8.0)	2.05±0.335	0.79	74.19	31.05	0.83	0.123 (0.85 - 0.17)	1.58±0.28	0.04	50.07	98.00
Permethrin	92.65 (50.9 - 130.2)	1.70±0.394	0.05	874.06	0.6	0.997	5.15 (3.4 - 7.3)	1.41±0.27	0.02	17.99	94.44
Fenvalerate	23.169 (15.7 - 32.4)	1.38±0.217	0.51	133.92	22.39	0.881	0.589 (0.36 - 0.88)	1.1±0.20	0.07	39.34	97.46
Es-fenvalerate	8.29 (5.7 - 11.9)	1.32±0.21	0.4	89.14	28.86	0.843	0.257 (0.169 - 0.370)	1.38±0.269	0.02	32.26	96.90
Fenpropathrin	68.36 (50.2 - 89.2)	1.98±0.31	0.42	182.78	4.77	0.976	3.275 (1.93 - 4.84)	1.257±0.265	0.01	20.87	95.21

Table 4: Toxicity of the insecticides with PBO to Sharkia 4th instar *S.littoralis*.

Pesticide name	LC_{50} (Limits)	Slope±SE	H	RR	M	Gf	LC_{50}+ PBO	Slope±SE	H	SR	(%) S
Lambdacyhalothrin	4.548 (2.3 - 7.3)	1.52±0.23	1.16	63.16	34.71	0.81	0.23 (0.11 - 0.35)	1.378±0.29	0.09	19.77	94.94
Cypermethrin	11.7 (7.635 - 15.84)	1.784±0.314	0.92	128.57	5.28	0.97	0.26 (0.18 - 0.37)	1.49±0.273	0.14	45.00	97.78
α -cypermethrin	1.289 (0.89 - 1.8)	1.42±0.217	0.1	3.03	90.26	0.31	0.074 (0.021 - 0.13)	1.7±0.30	1.29	17.42	94.26
Delta-methrin	0.337 (0.24 - 0.45)	1.59±0.227	0.16	4.06	94.8	0.23	0.005 (0.003 - 0.007)	1.58±0.29	0.12	67.40	98.52
Permethrin	14.38 (10.3 - 19.7)	1.51±0.221	0.38	135.66	16.58	0.91	1.11 (0.79 - 1.47)	1.8±0.269	0.69	12.95	92.28
Fenvalerate	17.82 (13.1 - 23.8)	1.59±0.195	0.11	103.0	24.39	0.87	2.21 (1.5 - 3.1)	1.48±0.27	0.01	8.06	87.60
Es-fenvalerate	7.146 (5.0 - 10.2)	1.27±0.173	0.19	76.84	31.68	0.83	0.195 (0.13 - 0.28)	1.32±0.21	0.31	36.65	97.27
Fenpropathrin	27.39 (17.9 - 37.5)	1.64±0.294	0.57	73.23	23.17	0.88	0.61 (0.44 - 0.83)	1.59±0.23	0.99	44.90	97.77

Table 5: Toxicity of the insecticides with PBO to Fayoum 4th instar *S.littoralis*.

Pesticide name	LC_{50} (Limits)	Slope±SE	H	RR	M	Gf	LC_{50}+ PBO	Slope±SE	H	SR	(%) S
Lambdacyhalothrin	13.8 (8.7 - 19.3)	1.529±0.285	0.22	191.67	12.02	0.9379	0.235 (0.16 - 0.32)	1.57±0.23	0.09	58.72	98.30
Cypermethrin	25.84 (18.3 - 34.6)	1.76±0.29	0.12	283.95	1.43	0.9928	0.378 (0.22 - 0.58)	1.05±0.20	0.44	68.36	98.54
α -cypermethrin	1.02 (0.56 - 1.6)	1.01±0.20	0.39	2.4	84.82	0.3896	0.2 (0.14 - 0.27)	1.7±0.29	0.72	5.10	80.39
Delta-methrin	2.32 (1.4 - 3.4)	1.3±0.27	0.14	27.94	59.49	0.6364	0.117 (0.08 - 0.17)	1.268±0.21	0.22	19.82	94.95
Permethrin	19.97 (11.7 - 28.67)	1.399±0.277	0.55	188.39	13.57	0.9296	0.516 0.34 - 0.71)	1.53±0.23	0.21	38.70	97.42
Fenvalerate	13.19 (7.6 - 19.5)	1.18±0.213	0.85	76.24	35.88	0.8	0.31 (0.16 - 0.47)	1.21±0.22	0.32	42.55	97.65
Es-fenvalerate	5.479 (2.38 - 8.0)	1.697±0.421	0.8	58.914	33.1	0.8179	0.7 (0.46 - 1.03)	1.195±0.20	0.05	7.83	87.22
Fenpropathrin	28.73 (18.5 - 41.0)	1.397±0.27	0.19	76.82	25.71	0.8619	1.64 (1.12 - 2.24)	1.64±0.287	0.28	17.52	94.29

PBO has been shown to inhibit both cytochrome P_{450} monooxygenases and esterases in Australian *H. armigera*. Kennaugh *et al.*, 1993 found that piperonyl butoxide eliminated high resistant levels in *Helicoverpa armigera* strain that showed a 20-fold to permethrin and moderate levels of permethrin detoxification in the resistant strain and a lower rate of permethrin. Result of bioassays and field trials on *Spodoptera littoralis* in Egypt revealed that There are many factors illustrate the control failure in the field such as that the synthetic pyrethroids Fenvalerate, Cypermethrin and Decamethrin have a high intrinsic activity, but a low activity against field populations. A reason for this is seen in their lacking penetration into the leaf, resulting in poor control of early instars (Buholzer, and Mabrouk, 1982).

The Percent of resistance suppression (%S) were intended to persuade about how much LC50 decreased after uses of PBO to each insecticide. Evenly all insecticide were between 95.21 and 99.6% resistance suppression except for α -cypermethrin in Fayoum was 80%. This suggest that When OP's resistance seems to be unstable and become not effective rapidly, the use of pyrethroids always then needed and piperonyle butoxide may have stop pyrethroid resistance when it happened. But when piperonyl butoxide becomes ineffective, we can try to use OP's compounds again.

Many curiosities in the use of synergist to reduce the resistance incidence by combined applications among the new generation of pyrethroids were projected. piperonyl butoxide (PBO) is an effective synergist for synthetic pyrethroids due to its ability to inhibit the monooxygenases as detoxifying enzymes Recently, Pasay *et al.* 2009, determine the role of metabolic degradation as a mechanism for acaricide resistance by test for synergistic activity of PBO with permethrin in a bioassay of mite killing and the inhibition of cytochrome P_{450} monooxygenase activity (81%) with PBO, then metabolic resistance can be completely negated by PBO. The failure of synergists that block the degradation of pesticide to overcome resistance in the field, the relative importance of detoxification, and the changes in the site of actions is due to the selection of the resistance genes in the population (Metcalf, 1967) as well as by kind of selection pressure.

The high gene frequencies (GF) for the pyrethroids, as well as the low RRs and SRs, indicate that resistance levels were very high in those strains (Tables 2, 3, 4 and 5). There are many factors affect the rise of the insecticide resistance and gene frequencies: (1) Species with large population sizes can also depend on gene flow to more time is required for the frequency of adaptive allele to increase. (2) Movement of rare alleles into new populations will also be delayed as weak selection pressure for the new phenotype. (3)When the selection pressure was not applied, a gradual decline in the frequency of R gene then occurred, the lower reproductive potential, and other factors may explain the differences in fitness evidence (refugia) or delaying the evolution of resistance Gorghiou and Tailor (1977). (4) Effective dominance and phenotypic expression under field conditions also play role in the development of resistance management Mason *et al.*, (1989). These are providing exciting insight in to the homology of resistance mutations between species and the frequency with which they arise (Tabashnik, 1990 and Ffrench Constant *et al.*, 1996). The role of synergists

in resistance management is related directly to an enzyme-inhibiting action, restoring the susceptibility of insects to the chemical, which would otherwise require higher levels of the toxicant for their control. For this reason synergists are considered straightforward tools for overcoming metabolic resistance, and can also delay the manifestation of resistance Bernard, and Philogène 1993.

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مستويات المقاومه الحاليه للمبيدات البيريثرويديه في دوده ورق القطن

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بيبرونيل بيوتوكسيد والذي هو الماده الكيماويه الاخري التي اذا اضيفت للمبيد فانها تنشطه ابلديا وتزيد من فعاليته اذا يعتبر من منشطات المبيدات. ونظرا للزياده المستمره في مستويات المقاومه لدوده ورق القطن عامه للمبيدات فان المنشطات تعتبر من المفاتيح الهامه لزياده الفعاليه للمبيدات والتي فقدت قدرتها علي مقاومه الافه. لذا تم عمل حصر لفعاليه المنشط بيبرونيل بيوتوكسيد في تنشيط المبيدات البيريثرويد والتي اظهرت مقاومه عاليه في معظم المناطق المزروعه بالقطن في الجمهوريه. وعن طريق عمل البيواساي علي العمر الرابع لدوده القطن تم الحصول علي خطوط السميه والجرعات النصفيه لهذه المبيدات بمفردها او بعد التنشيط وعمل المقارنه بينهما. اوضحت النتائج ان المنشط المختبر نجح في تنشيط معظم المبيدات التي تم اختبارها فيما عدا مبيدي الايسفينفاليرات والفاسبيرميثرين في الفيوم والفينفاليرات في الشرقيه بعد الخلط اعطي نسبه تنشيط منخفضه. ويمككتنا القول ان نسب كبت قيم المقاومه والناتجه عن التنشيط كانت عاليه في كل المبيدات فيما عدا مبيد الالفاسبيرميثرين في الفيوم.

من كل النتائج يتضح لنا انه يمكن استخدام المبيدات البيريثروديه في حاله ظهور حالات من مقاومه الافه للمبيدات الفوسفوريه اما اذا اظهرت ايضا الافه مقاومه للمبيدات البيريثويد فانه لابد من تنشيطها بالمنشطات المختلفه وخاصه البيبرونيل بيوتوكسيد اما اذا اصبح غير فعال فانه دائما ينصح بالرجوع الي استخدام المبيدات الفوسفوريه مره اخري.

قام بتحكيم البحث

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