EVALUATION OF ONE BIOAGENT AND AN INSECT GROWTH REGULATOR AGAINST THE COTTON LEAFWORM USING PCR

ABDEL-WAHED, M. S.[1], SAADIA M. EL-BERMAWY, [2], K. A. EL-DOUGDOUG[1], AMAL K. MABROUK[3] and MARWA M. ABDEL-AZIZ [3]

1. *Faculty of Agriculture, Ain Shams University.*
2. *Faculty of Education, Ain Shams University.*
3. *Plant Protection Research Institute, ARC.*

(Manuscript received 3 July 2012)

Abstract

This study was carried out on 6th instar larvae of *Spodoptera littoralis* treated in 2nd instar larvae with LC_{50} of Match and Protecto at 0.0057 ppm, and 0.1099 gm/ml., respectively, Five random primers were used in this study to generate a fragmenting pattern as a tool to investigate the molecular differences between treated samples and control. The numbers of unique and common fragments generated by using these primers (OPO1, OPO2, OPO3, OPO4 and OPO5) was recorded. It has been found that primer OPO2 was the most powerful in generating a unique informative fragmenting pattern, it gave 4 specific unique fragments. The primer OPO4 was the poorest one in generating an informative fragmenting pattern.

INTRODUCTION

The Egyptian cotton leaf worm, *S. littoralis* (Boisd.), (Lepidoptera: Noctuidae) is an important pest in Egypt and other countries in Africa and Asia causes extensive economic losses in many cultivated crops (Frank *et.. al.,* 1990). The extensive use of insecticides for controlling *S. littoralis* (Boisd.) caused negative effects on humans, living organisms and environment (Chantelli-Forti *et.. al.,* 1993 and Chaudhuri *et. al.,* 1999). Furthermore, this insect acquired resistance to various classes of insecticides (Denholm *et. al.,* 1998). The problems and hazards that have arisen as a result of using conventional insecticides were incentives for the search of alternative control agents. Microbial control agents are a primary means of biological control for insect pests. The use of microbial control agents is targeted for a particular pest species. The entomopathogens that have been used in biological control include representatives of bacteria, fungi, viruses, nematodes, protozoa and insect growth regulator (Dent, 2000). To evaluating the differences between treated and non treated larvae using the RAPD-PCR technique El Gohary *et. al.* (2000).

MATERIALS AND METHODS

1-Rearing technique of the Egyptian cotton leaf worm, *S. littoralis* (Boisd)

The original insect culture was obtained from the Research Division of the Cotton Leaf worm, Plant Protection Research Institute. Newly hatched larvae were transferred to clean glass jars covered with muslin held in position with rubber fragments and incubated under laboratory condition at 27º±2ºC, 60 ± 5% RH, and 8:16 LD photoperiod. They were fed on castor oil leaves and examined daily. Upon pupation, pupae were collected, sexed and emerged moths were placed in pairs in breeding glass globes. These globes were supplied with leaves of tafla, *Nerium oleander* (L.) as an oviposition site.

2-Compounds tested

The potency of one insect growth regulator and one bioagents were evaluated for their effect on *S. littoralis* larvae:-

2.1. Lufenuron is an insect growth regulator which acts as a chitin synthesis inhibitor, with the trade name Match® 5%.

This chemical was obtained from Syngenta Agro S.A.E.

2.2. *Bacillus thuringiensis* var. *kurstaki* with the trade name Protecto®.

The above mentioned microbial agent was obtained from Plant Protection Research Institute Biopesticide Unit Production.

3. Bioassay

The insecticidal activity of the two chemicals was assessed on newly ecdysed 2nd instars of *S. littoralis* larvae.

a) Match: A series of aqueous concentrations were prepared which were 0.25, 0.1, 0.05, 0.025, 0.0125, 0.00625 and 0.003125 ppm.

b) Protecto: A series of dilution were prepared from 1 gm of the product obtained as a wettable powder, 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 gm/ml.

Treatment of larvae was conducted by the leaf dipping technique, a fresh castor oil leaves, *R. communis* (L) were cleaned and immersed for 10 seconds in one of the prepared concentrations or dilutions of each one tested chemical. The treated leaves were left to dry at room temperature before being offered to newly ecdysed 2nd instars *S. littoralis* larvae. Larvae were offered treated leaves for 24 hr and subsequently larvae were fed on untreated castor oil leaves for the following duration of the larval stage. Each treatment comprised 20 larvae and was replicated three

times. The same number of larvae was considered as a control in which larvae were offered castor oil leaves dipped in water.

4- The Molecular study

The DNA was extracted according to the method of Sambrook *et. al.* (1989). Before any analysis, it was important to determine the concentration and purity of isolated DNA, this was carried out by estimating UV absorbance at wave length of 260 and 280 nm using a spectrophotometer. DNA was subjected to PCR in order to generate the fragmenting profile. The random primers used were OPO1, OPO2, OPO3, OPO4 and OPO5. Reactions were carried out in a thermocycler (Progeny 30, Techno, Cambridge Ltd. Dux ford Cambridge, UK). The PCR profile was as follows: 94 ºC for 5 min, 94 ºC for 1 min, 40 ºC for 1min, 72 ºC for 2 min, and final extension at 72 ºC for 7 min. Then the PCR reaction was kept at 4 ºC over night, till migration on agrose was occurred.

NO	Primer	Sequence
1	OPO1	5'- GGC ACG TAA G -3'
2	OPO2	5'- ACG TAG CGT C -3'
3	OPO3	5'- CTG TTG CTA C -3'
4	OPO4	5'- AAG TCC GCT C -3'
5	OPO5	5'- CCC AGT CAC T -3'

The gel was prepared with wells into which the DNA fragments are added and subemerged under an electrolyte buffer solution between a positive and a negative electrode. The DNA fragments are negatively charged so the wells containing them are placed closest to the negative electrode. When the current is turned on the DNA moves through the pores in the gel towards the positive electrode. PCR- DNA marker was used to determine the M.w of each fragment. The shorter fragments move faster because they are able to move through the pores of the gel more easily, whereas the longer DNA fragments move more slowly through the pores (Hurlbert, 1999).

5- Statistical analysis

1- Results were presented graphically as log/probit regression lines, and toxicity LC_{50} and LC_{90}values as well as the slope according to Finney, (1971) using "LdPLine$^{®}$" software.

2- DNA sequences were analyzed using version 6 of the Gel-Pro Analyzer package of genetics computer program.

RESULTS AND DISCUSSION

1- Bioassay Test

The efficiency of the two tested compounds, the IGR Match and formulated bioagent, Protecto (*Bacillus thuringiensis* var. *kurstaki*), were evaluated on 2nd instar larvae of *S. littoralis* (Boisd.).

The IGR Match toxicity had a strong effect on 2nd instar larvae giving the LC_{90} and LC_{50} 0.0434 and. 0.0057 ppm, respectively. As in (Table 1).

Meanwhile the LC90 and LC_{50} of Protecto were 0.6549 and 0.1099 gm/ml., respectively. As in (Table 1).

Table 1. Susceptibility of *S. littoralis* 2nd instar larvae to Match and Protecto.

Compound	Unit	LC_{90}	LC_{50}	Slope
Match	ppm.	0.0434	0.0057	1.4599±0.2411
Protecto	Gm/ml.	0.6549	0.1099	1.6531±0.2165

2- Molecular Studies

This study has been carried out on 6th instar larvae of *S. littoralis* which treated in 2nd instar larvae with LC_{50} of Match and Protecto at 0.0057 ppm, and 0.1099 gm/ml., respectively.

Five random primers were used (OPO1, OPO2, OPO3, OPO4 and OPO5.) to generate the specific by which an informative conclusion could be summarized. The five primers used are shown in table (2) and fig. (1) along with their sequences.

Using primer OPO1, a distinguishing pattern was obtained when using a control, Match treated, and Protecto-treated insects (larvae) as a source of DNA.

In this primer, the treatment with Match induced the generation of a fragment with size of 979 bp. this fragment was absent in both control and Protecto treatment, the same finding also in Match and Protecto regarding the induction of a fragment with a size of 452 bp and also this fragment was absent in control. On other hand, missing fragments were noticed in both Match and Protecto while this fragment in size of 251 was present in only control.

The similarity index (S.I.) was recorded between Match, Protecto treated samples and their control was (0.70 –0.66) respectively, as recorded in Table (3).

In primer OPO2, the treatment with both tested compounds resulted in the presence of two fragment with size of 350 and 274bp in Protecto This fragment was absent in control and Mach, that may indicated this action of Protecto. The same also

we found a fragments with size of 612 and 408bp were present in both control and Protecto but this fragment was absent in Match.

The similarity index (S.I.) was recorded between Match, Protecto treated samples and their control was (0.63 –0.85) respectively, as recorded in Table (4).

In primer OPO4, the treatment with Protecto resulted in the absence of a fragment which was present in both control and Match, this absence may be attributed to the treatment with Protecto. However, for the same treatment a fragment with size 570 bp was noticed while this fragment was absent in both control and Match. Two fragments with size 358 bp were absent in the treatment in both Match and Protecto a fragment was observed in the control. However, the treatment with Protecto led to the absence of a fragment and these fragment was present in both control and Match with size of 959 bp.

The similarity index (S.I.) was recorded between Match, Protecto treated samples and their control was (0.94 –0.77) respectively, as recorded in Table (5).

In primer OPO5, two fragments were noticed due to the treatment with Match, while, the same molecular size fragments were present in both control and Protecto treatment with size (1461,1021,839,431bp).

On the other hand, a specific unique fragment was obtained (at molecular size of 859bp) due to the treatment with Match. While this fragment was absent in the control and Protecto.

The similarity index (S.I.) was recorded between Match, Protecto treated samples and their control was (0.25 –0.83) respectively, as recorded in Table (6).

In primer OPO3, a missing fragment was detected in the treatment with the two treatments (Match and Protecto) while resemblance fragment was presented with control at size of 983 bp. The Protecto treatment resulted in a fragment that present only in this lane with size of 1817 bp and this fragment was absent in both control and Match treatment.

On the other hand, the treatment with both Match and Protecto resulted in the absence of a fragment with M.w. of 322bp which was observed only in control.

The similarity index (S.I.) was recorded between Match, Protecto treated samples and their control was (0.86 –0.75) respectively, as recorded in Table (7).

RAPD-PCR technique shows the DNA diversity among the 6th instar larvae of *S. littoralis* which was treated as 2nd instar larvae with LC_{50} of Match and Protecto. 52 DNA fragments were detected using five random primers. 22 fragments were common in treated and untreated larvae of *S. littoralis*, they represent 42.3 % of all detected fragments (table 8).

On the other hand the RAPD-PCR technique shows 14 polymorphic amplified fragments represented 26.9%. This ratio is due to treatment with Match and Protecto (table 8).

Treated and untreated larvae showed 16 unique fragments that represented 30.7 % of all detected fragments (table 8).

Finally the Match has more effective on DNA generated than Protecto.

The previous results showed that primers number (OPO2) was the powerful one in generating a unique informative fragmenting pattern, it give four specific unique fragments. While the primer OPO4 was the poorest one in generating an informative fragmenting pattern as it gives seven common fragments this results agree with those reported by El Gohary *et. al.* (2000), Abd EL- Aziz, (2006) and Abdel- Ghany (2011) .
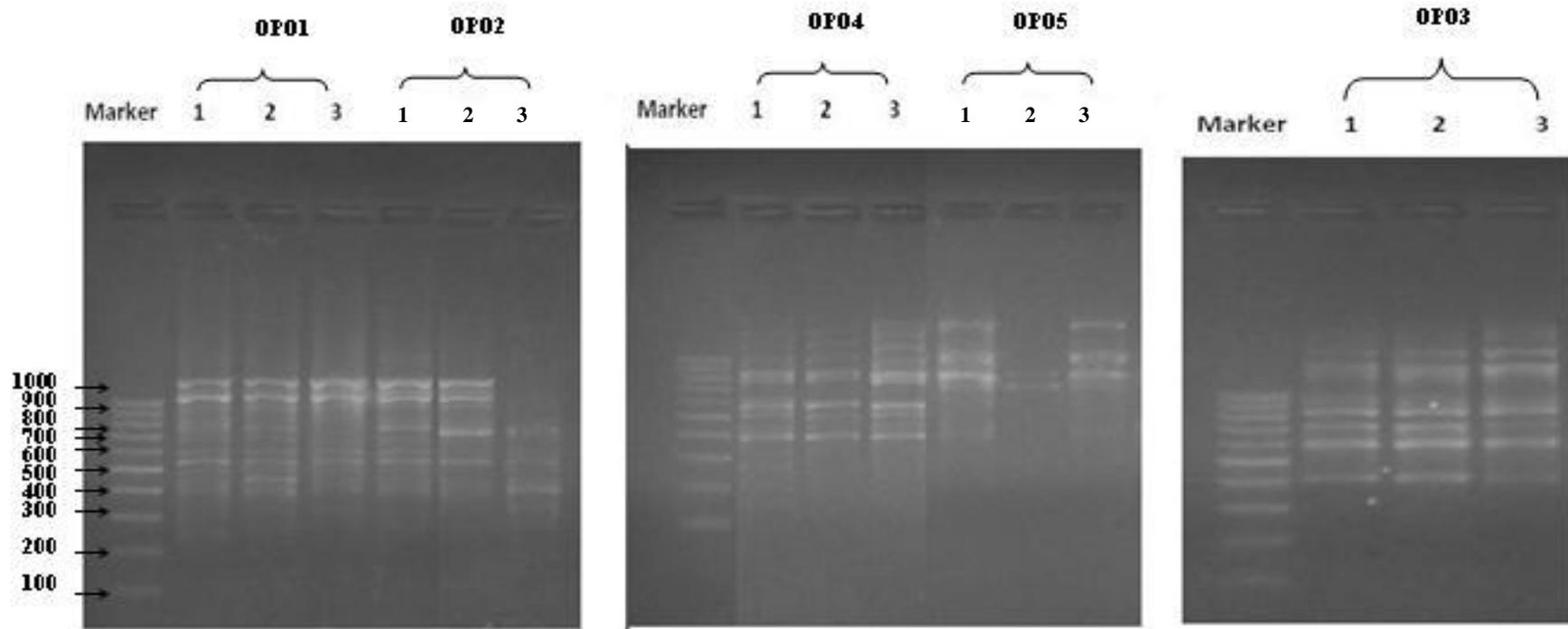
Fig. 1. Molecular fingerprinting using RAPD DNA for pattern for samples treated with Match, Protecto and control

M=Marker   1=Control   2=Match   3=Protecto

Table 2. RAPD-PCR Products in 6th instar larvae of *Spodoptera littoralis* which treated in 2nd instar larvae with LC_{50} of Match and Protecto compared with control using random primers.

Lanes	Primer 1: OPO1									Primer 2 : OPO2									Marker	
Rows	Control			Match			Protecto			Control			Match			Protecto				
	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount
r1										1694	8.687	0.514								
r2	1288	11.774	0.561	1288	13.863	0.561	1288	12.851	0.561	1261	13.384	0.564	1261	15.719	0.564					
r3	1088	12.943	0.589	1088	14.632	0.589	1065	15.08	0.593	1065	18.846	0.593	1065	16.966	0.593				1000	8.936
r4				979	11.937	0.607							979	7.51	0.607				900	5.779
r5	785	14.526	0.645	785	14.743	0.645	849	13.082	0.631	770	12.638	0.648							800	6.888
r6	713	8.342	0.662				713	9.681	0.662	687	9.303	0.669	727	23.61	0.659	741	23.33	0.655	700	7.85
r7	612	8.303	0.69	624	9.616	0.686	600	6.956	0.693	612	6.707	0.69				600	9.897	0.693	600	9.834
r8	548	8.665	0.711	548	14.574	0.711	548	16.489	0.711	548	9.442	0.711	548	16.145	0.711	538	10.131	0.714		
r9										500	5.136	0.729				509	8.204	0.725	500	11.447
r10				452	9.806	0.746	452	7.844	0.746	452	6.348	0.746	434	20.195	0.754					
r11	408	14.58	0.764	400	10.857	0.768	400	9.839	0.768	408	9.875	0.764				400	21.822	0.768	400	11.639
r12	324	11.88	0.807				343	8.214	0.796							350	18.137	0.793		
r13																			300	13.479
r14	251	9.025	0.85													274	8.483	0.836		
r15																			200	11.713
r16																			100	12.437
r17																				
r18																				

Continued Table (2)…………

Rows	Primer 3 : OPO4 Control M.w	amount	Rf	Match M.w	amount	Rf	Protecto M.w	amount	Rf	Primer 4 : OPO5 Control M.w	amount	Rf	Match M.w	amount	Rf	Protecto M.w	amount	Rf	Marker M.w	amount
r1							1730	9.552	0.15	1461	19.192	0.138				1461	17.972	0.138		
r2	1624	7.471	0.169	1658	8.562	0.163	1557	8.699	0.181							1209	7.1509	0.194		
r3	1431	9.116	0.206	1461	12.309	0.2	1372	10.504	0.219	1021	17.974	0.244				1021	19.612	0.244		
r4	1235	7.751	0.25	1235	14.43	0.25	1209	10.803	0.256				859	41.432	0.294					
r5										839	17.21	0.3				839	20.326	0.3		
r6	1021	10.47	0.306	1043	13.549	0.3	1021	20.17	0.306	719	10.406	0.338	719	58.57	0.338	700	15.027	0.344	1000	8.351
r7	959	8.57	0.325	959	7.657	0.325				613	16.138	0.381							900	4.89
r8																			800	7.282
r9	716	15.751	0.406	716	15.934	0.406	716	13.1661	0.406	400	19.089	0.494				431	20.011	0.475	700	7.182
r10	641	8.651	0.438	641	11.636	0.438	655	8.023	0.431											
r11							576	6.232	0.469										600	8.299
r12	510	20.8	0.506	510	15.938	0.506	510	12.857	0.506											
r13																			500	9.861
r14																			400	13.214
r15	358	11.446	0.606																	
r16																			300	15.3
r17																			200	11.508
r18																			100	14.138

Continued Table (2)………….

Lanes	Primer 5 : OPO3									Marker	
	Control			Match			Protecto				
Rows	M.w	amount	Rf	M.w	Amount	Rf	M.w	amount	Rf	M.w	Amount
r1							1817	10	0.211		
r2	1579	7.998	0.253	1579	11.822	0.253	1579	9.642	0.253		
r3	1348	14.704	0.3	1301	22.823	0.31	1348	19.163	0.3		
r4	983	8.509	0.39							1000	8.858
r5	849	13.223	0.432	849	13.983	0.432	865	15.755	0.426	900	6.25
r6										800	7.142
r7	727	10.438	0.474	713	13.744	0.479	741	9.819	0.468	700	7.035
r8	600	19.724	0.532	600	22.972	0.532	610	20.144	0.526	600	7.939
r9										500	12.217
r10											
r11	415	16.134	0.635	423	15.302	0.63	415	15.513	0.635	400	12.245
r12	322	9.306	0.705							300	12.936
r13										200	11.339
r14										100	14.122
r15											
r16											
r17											
r18											

Table 3. Similarity index (S.I.) and genetic distance (G. d.) between treated samples with Match, Protecto and untreated 2nd larval instar of *S. littoralis* using Primer (1)*.*

S.I.

G. d.

Samples	Control	Match	Protecto
Control	-	0.70	0.66
Match	0.30	-	0.82
Protecto	0.34	0.19	-

Table 4. Similarity index (S.I.) and genetic distance (G. d.) between treated samples with Match, Protecto and untreated 2nd larval instar of S. littoralis using Primer (2) .

S.I.

G. d.

Samples	Control	Match	Protecto
Control	-	0.63	0.85
Match	0.37	-	0.30
Protecto	0.15	0.7	-

Table 5. Similarity index (S.I.) and genetic distance (G. d.) between treated samples with Match, Protecto and untreated 2nd larval instar of S littoralis using Primer (3) .

S.I.

G. d.

Samples	Control	Match	Protecto
Control	-	0.94	0.77
Match	0.06	-	0.82
Protecto	0.23	0.18	-

Table 6. Similarity index (S.I.) and genetic distance (G. d.) between treated samples with Match, Protecto and untreated 2nd larval instar of S littoralis using Primer (4) .

S.I.

G. d.

Samples	Control	Match	Protecto
Control	-	0.25	0.83
Match	0.75	-	0.25
Protecto	0.17	0.75	-

Table 7. Similarity index (S.I.) and genetic distance (G. d.) between treated samples with Match, Protecto and untreated 2nd larval instar of S littoralis using Primer (5) .

S.I.

G. d.

Samples	Control	Match	Protecto
Control	-	0.86	0.75
Match	0.14	-	0.86
Protecto	0.25	0.14	-

Table 8. DNA diversity among S. littoralis treated with Match and Protecto using RAPD-PCR.

Primers	Polymorphism				Genetic markers (bp)*		
	TAF	MAF	PAF	Unique	Control	Treated with Match	Treated with Protecto
OPO1	11	6	3	2	251	979	-
OPO2	13	2	6	5	1694 -770	979	350 - 274
OPO4	11	7	1	3	358	-	1730 - 576
OPO5	8	1	4	3	613	859	1021
OPO3	9	6	-	3	983 - 322	-	1817
Total	52	22	14	16	7	3	6

bp---------------- size of genetic marker (unique).
TAF-------------- total amplified fragments.
MAF------------ monomorphic amplified fragments (common).
PAF------------- polymorphic amplified fragments.

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تقييم فاعلية مركب حيوي ومنظم نمو حشري ضد دودة ورق القطن باستخدام تقنيه التوصيف الجزيئي

محمد سالم عبد الواحد[1] ،   سعدية محمد البرماوى[2] ،   خالد عبد الفتاح الدجدج[1] ،
آمال كامل مبروك[3] ،   مروة محمد محمود[3]

١. كلية الزراعة – جامعة عين شمس.

٢. كلية التربية –جامعة عين شمس.

٣. معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقى – جيزة .

اجريت هذه الدراسة علي يرقات العمر السادس لدوده ورق القطن التي عوملت في العمر الثاني بالتركيز النصف مميت لكلا من مركبي الماتش والبرتكتو والتي كانت 0.0057 جزء في المليون و 0.1099 جرام/مللي على التوالي. تم استخدام خمسة بادئات عشوائية (OPO1, OPO2, OPO3, OPO4 , OPO5) لإنتاج نموذج حزمى مميز كآدة لدراسة التباينات الجزيئية بين مختلف المعاملات. وتم حصر وعد الحزم المميزة والحزم المشتركة التى تم انتاجها بواسطة استخدام هذه البادئات الخمس، وقد وجد أن البادئ OPO2 هوأقوى البادئات فى انتاج حزم مميزة معبرة، حيث أعطى 4 حزم مميزة متفردة بينما كان البادىء OPO4 أقل البادئات المستخدمة قدرة على انتاج حزم معبرة.