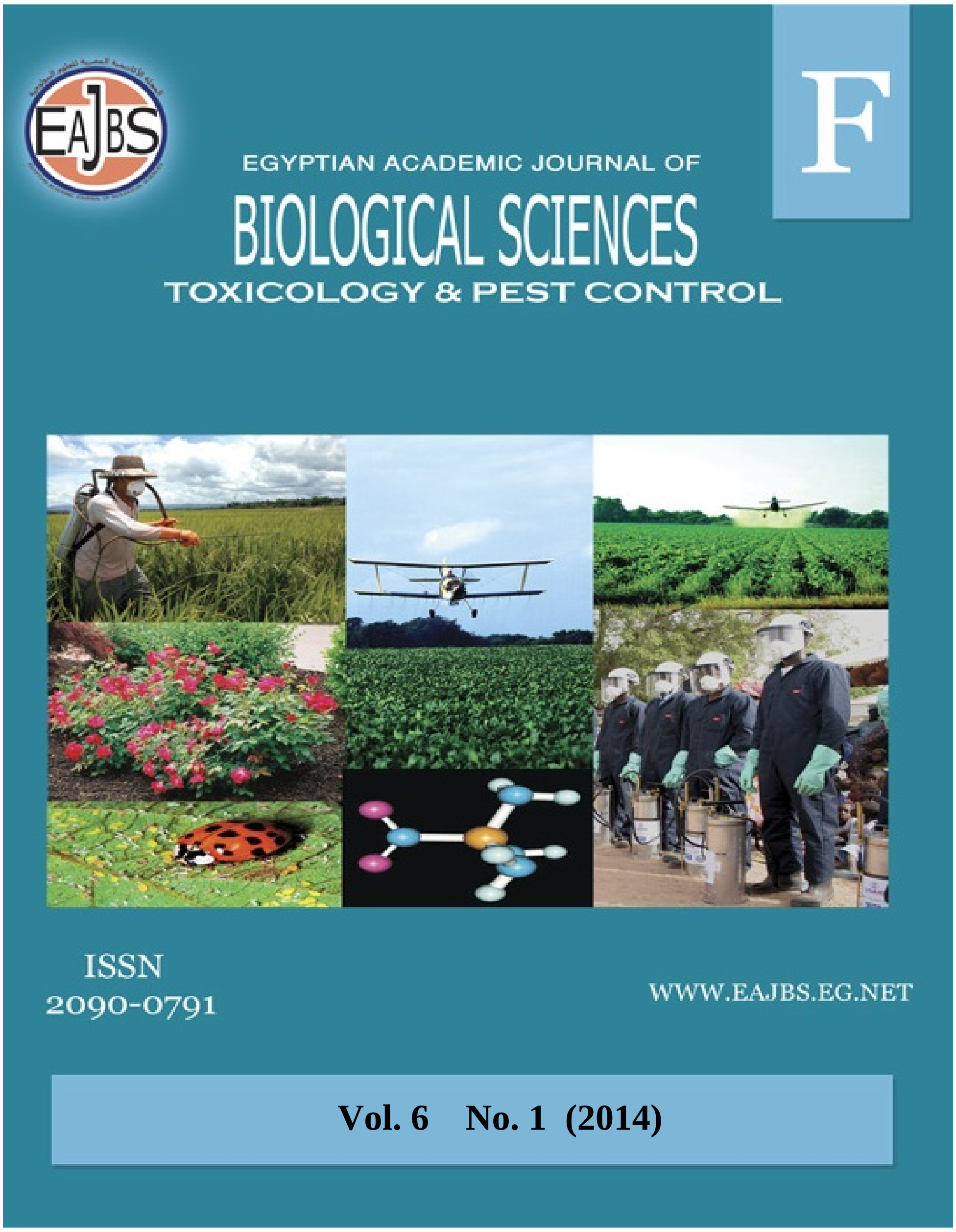

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Monitoring of resistance to biocides against cotton leaf worm *Spodoptera littoralis* (Boisd.) during 2012 to 2014 cotton seasons in Egypt

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ABSTRACT

Field strains of the cotton leafworm *Spodoptera littoralis* (Boisd.) were collected from three Governorates (Gharbya, Kafr El-Sheikh and Behera) during 2012, 2013 and 2014 cotton seasons for monitoring of its resistance to tested biocides.

The results indicate that resistance ratios RR and RC fluctuated from one year to another and from Governorate to another. During three cotton seasons the tested *Bt* (Dipel 2x, Dipel DF, Agerine and Protecto) showed high levels of resistance in all Governorates during 2014 cotton season which (RC) reached (13.93, 9.23 and 15.04), (9.24, 10.55 and 17.93), (6.01, 8.12 and 14.48) and (6.95, 7.08 and 8.34) in Behera, Gharbya and Kafr El-Sheikh respectively. Also, RC for Radiant compound reached to (14.07, 11.58 and 22.05) in the same Governorates. While Radical recorded medium levels of resistance (2.16, 2.29 and 4.07) and Spintor had low levels of resistance (1.21, 1.73 and 1.66) to the same field strains during cotton season 2014.

The previous results suggest that Biocides Radical and Spintor may be recommended as an effective component of the future IPM programs against *Spodoptera littoralis* on cotton fields.

INTRODUCTION

Developing integrated pest management (IPM) systems that build on the natural control agents already present in an agro- ecosystem require a thorough understanding of the biological agents present and their interactions with their host pest species and the crop plant. Biocides because their selectivity is well suited for being key components in such system because of their lack direct activity on natural enemies.

For several decades since its discovery, formulations of biocides have been seen as the ideal means of controlling Lepidopteran pests in agriculture because of the many attributes that differentiate this microbial insecticide from the synthetic chemical formulations. No toxicity to mammals, environmental friendliness, good integration with other pest control methods and the possibility of being mass produced at farm level, at low cost, all made the biocides the much needed tool for IPM programmers.

Bacillus thuringiensis crystal proteins are preferred and widely used as an alternative to chemical pesticides in pest management strategies against insect pests of agriculture crops (Roh *et al*., 2007). Also, spintor is an insecticide based on a fermentation product of the soil bacterium actinomycete *Saccharopolyspora Spinosa*. It exhibits a high degree of selective toxicity towards the insect order Lepidoptera , but less toxic to many beneficial arthropods (Thompson *et al*., 2000).

The purpose of the present study is to investigate the resistance of tested biocides against the cotton leafworm *Spodoptera littoralis* (Boisd.) collected from different Governorates of Lower Egypt in cotton fields during 2012, 2013 and 2014 seasons.

MATERIALS AND METHODS

A: Field strains:

Three field colonies of the cotton leafworm *S. Littoralis* were collected from the cotton fields in several locations Gharbya, Behera and Kafr El- Sheikh during 2012, 2013 and 2014 cotton seasons.

After collection, the egg-masses were kept separately in 400 ml Jars, covered with muslin held in position by rubber band until the eggs hatched. The jars were provided with costar-oil leaves for larval feeding and to provide the required humidity for hatching. All cotton leafworm field and laboratory strains were reared at 25± 2^{o}C and 70% ±5% relative humidity.

B: Biocides used:

1- Dipel 2x 6.4%WP (*Bt* subsp. Kurastaki) was used at 200 gm/fed
2- Dipel DF 6.4%WG (*Bt* subsp. Kurastaki) was used at 200 gm/fed
3- Protecto 9.4%WP (*Bt* subsp. Kurastaki) was used at 300 gm/fed
4- Agerine 6.5%WP (*Bt* subsp. Seravora egypti.) was used at 250 gm/fed
5- Radical 0.5% EC (Emamectin benzoate) was used at 200 gm/fed
6- Radiant 12% SC (Spinetoram) was used at 25 ml/fed
7- Spintor 24% SC (Spinosad) was used at 50 ml/fed.

C: Method of Applications

The LC_{50} and LC_{95} values of the tested biocides were determined by applying the leaf dipping technique. Seven different concentrations from each biocide were used. Each concentration was replicated five times and each replicate contains 20 second instar larvae. After 72 hr. mortality counts were recorded.

D: Statistical analysis:

Abbott's formula (Abbott, 1925) was adapted to correct the mortality data for natural mortalities in the control. The corrected percent mortalities were statistically computed according to (Finney, 1971). The rates of resistance were expressed as resistance ratio (RR) at the level of the field strains as compared with the lab-strain which has been reared in laboratory condition for more than ten generations without exposure to any insecticide.

Resistance ratio RR= LC_{50} of the field strain / LC_{50} of the lab-strain.

The resistance coefficient (RC) values were calculated as follows:

RC = LC_{95} / recommended field dose.

The following criteria for resistance assessment were assumed:

RC≤ 1 the lack of resistance
RC = 1.2 – 2 low resistance
RC = 2.1 – 5 medium resistance
RC = 5.1 – 10 high resistance
RC > 10 very high resistance (Joann *et al*., 2013)

The relative toxicity (R.T.) was calculated from LC_{95} values by assigning an arbitrary value of 1.0 for the least effective compound.

RESULTS AND DISCUSSION

Resistance ratio (RR) and resistance coefficient values (RC) of the tested biocides determined against three field strains of the cotton leafworm *Spodoptera littoralis* collected from Gharbya, Behera and Kafr el-Sheikh Governorates during 2012, 2013 and 2014 cotton seasons are shown in Tables 1,2 and 3.

Table 1: Monitoring of resistance to tested biocides against *Spodoptera littoralis* collected from Behera

Biocides	FR ppm	Seasons	LC_{50} ppm	LC_{95} ppm	Slope	RR	RT	RC
Dipel 2x	-	Lab- strain	25.27 (11.88 – 49.28)	90.31 (43.75 – 170.11)	1.37	-	-	-
	64	2012	62.92 (44.89 – 101.52)	385.19 (188.47 – 2515.61)	1.63	2.49	2.31	6.02
	64	2013	90.51 (65.15 – 125.73)	450.13 (264.97 – 1442.62)	1.84	3.58	1.98	7.03
	64	2014	147.98 (105.49 – 230.87)	891.35 (451.00 – 4824.33)	1.64	5.86	1.00	13.93
Dipel DF	-	Lab- strain	27.46 (15.24 – 45.64)	110.51 (69.32 – 290.75)	1.27	-	-	-
	64	2012	53.16 (32.53 – 81.47)	382 (198.07 - 1780.65)	1.49	1.94	1.55	5.97
	64	2013	87.44 (55.55 – 133.60)	562.59 (304.27 - 2992.24)	1.84	3.18	1.05	8.79
	64	2014	113.13 (74.03 – 172.89)	591.25 (309.57 – 2578.74)	1.54	4.12	1.00	9.24
Agerine	-	Lab- strain	48.55 (27.11 – 122.77)	197.33 (145.34 – 672.12)	1.01	-	-	-
	81.25	2012	75.14 (42.36 – 116.25)	345.31 (215.42 – 1052.04)	1.64	1.55	1.41	4.25
	81.25	2013	84.64 (54.13 – 118.64)	455.94 (232.88 – 3670.19)	2.09	1.74	1.07	5.61
	81.25	2014	88.88 (59.42 – 130.06)	488.31 (279.26 – 1566.06)	1.73	1.83	1.00	6.01
Protecto	-	Lab- strain	24.68 (9.01 – 99.15)	187.33 (146.22 – 622.01)	1.22	-	-	-
	141	2012	61.53 (40.31 – 90.69)	357.25 (200.99 – 1215.76)	1.68	2.49	2.74	2.53
	141	2013	64.56 (38.89 – 129.48)	731.22 (269.00 – 14043.01)	1.22	2.62	1.34	5.19
	141	2014	123.19 (77.19 – 200.25)	980.15 (463.17 – 6221.13)	1.42	4.99	1.00	6.95
Radical	-	Lab- strain	0.44 (0.17 – 0.82)	3.63 (1.59 – 10.790	1.66	-	-	-
	5	2012	1.18 (0.77 – 1.69)	7.39 (4.37 – 20.08)	1.61	2.68	1.46	1.48
	5	2013	1.62 (1.07 – 2.38)	9.25 (5.23 – 31.15)	1.68	3.68	1.17	1.85
	5	2014	1.87 (1.18 – 3.04)	10.80 (5.33 – 93.45)	1.69	4.25	1.00	2.16
Radiant	-	Lab- strain	12.81 (10.24 – 14.23)	73.45 (41.35 – 360.09)	1.91	-	-	-
	30	2012	46.88 (35.32 – 59.30)	191.21 (133.38 – 363.)	2.09	3.66	2.21	6.37
	30	2013	67.04 (49.72 – 91.58)	390.36 (224.79 – 1241.28)	1.68	5.29	1.08	13.01
	30	2014	84.85 (64.27 – 112.04)	422.03 (262.46 – 1068.15)	1.84	6.62	1.00	14.07
Spintor	-	Lab- strain	2.87 (0.99 – 6.79)	14.65 (8.17 – 54.43)	1.39	-	-	-
	60	2012	3.13 (1.99 – 4.38)	12.75 (7.95 – 38.84)	2.09	1.09	5.68	0.21
	60	2013	5.52 (3.85 – 7.800	25.36 (15.54 – 65.79)	1.94	1.92	2.86	0.42
	60	2014	11.44 (7.44 – 17.33)	72.48 (38.75 – 290.69)	1.59	3.99	1.00	1.21

F.R. = Field recommended dose RR= Resistance Ratio RC = Resistance Coefficient RT= Relative toxicity

Table 2: Monitoring of resistance to tested biocides against *Spodoptera littoralis* collected from Gharbya

Biocides	FR ppm	Season	LC_{50} ppm	LC_{95} ppm	Slope	RR	RT	RC
Dipel 2x	-	Lab- strain	25.27 (11.88-49.28)	90.31 (43.75-170.11)	1.37	---	---	---
	64	2012	30.65 (22.72-40.87)	153.52 (97.64-347.17)	1.83	1.21	3.85	2.39
	64	2013	57.89 (40.84-85.69)	440.59 (229.71-1652.46)	1.45	2.29	1.34	6.88
	64	2014	79.99 (55.84-114.61)	590.44 (322.59-1965.18)	1.48	3.17	1.00	9.23
Dipel DF	-	Lab- strain	27.46 (15.24-45.64)	110.50 (69.32-290.75)	1.27	----	-	----
	64	2012	53.71 (35.54-77.91)	291.64 (169.47 -896.50)	1.74	1.96	2.32	4.56
	64	2013	69.48 (42.92-107.07)	502.29 (257.41-2401.03)	1.49	2.53	1.34	7.85
	64	2014	135.77- (88.83-207.48)	675.21 (365.15-3591.77)	1.84	4.94	1.00	10.55
Agerine	-	Lab- strain	48.55 (27.11-122.77)	197.33 (145.34-672.12)	1.01	---	-	---
	81.25	2012	96.84 (61.26-158.19)	563.86 (277.41-4904.62)	1.68	1.99	1.17	6.94
	81.25	2013	97.13 (62.63-150.46)	571.59 (309.09-3041.25)	1.84	2.00	1.15	7.03
	81.25	2014	114.90 (75.18-175.66)	660.08 (339.38-3030.81)	1.54	2.37	1.00	8.12
Erotecto	-	Lab- strain	24.68 (9.10-99.15)	187.33 (146.22-622.01)	1.22	----	-	---
	141	2012	131.07 (81.94-266.55)	952.69 (387.80-26499.49)	1.49	5.31	1.16	6.76
	141	2013	199.39 (130.47-304.74)	991.71 (536.31-5275.51)	1.74	8.08	1.11	7.03
	141	2014	201.56 (133.96-339.87)	1100.42 (541.54-8940.11)	1.84	8.17	1.00	7.08
Radical	-	Lab- strain	0.44 (0.17-0.82)	3.63 (1.59-10.79)	1.66	---	-	---
	5	2012	0.74 (0.48-1.03)	5.06 (3.07-13.18)	1.53	1.68	2.26	1.01
	5	2013	1.11 (1.02-2.14)	7.59 (4.75-16.42)	1.46	2.52	1.51	1.52
	5	2014	1.51 (10.02-2.14)	11.44 (6.41-36.31)	1.53	3.43	1.00	2.29
Radiant	-	Lab- strain	12.81 (10.24-14.23)	73.45 (41.35-360.09)	1.91	---	-	----
	30	2012	25.93 (18.47-33.92)	151.83 (101.94-295.52)	1.67	2.02	2.29	5.06
	30	2013	52.26 (38.79-69.11)	337.69 (210.40-764.21)	1.79	4.08	1.03	11.26
	30	2014	66.65 (50.32-89.21)	347.32 (210.13-948.30)	1.58	5.20	1.00	11.58
Spintor	-	Lab- strain	2.87 (0.99-6.79)	14.65 (8.17-54.43)	1.39	---	-	---
	60	2012	3.86 (1.83-5.40)	19.41 (10.07-86.94)	1.54	1.34	5.36	0.32
	60	2013	7.52 (5.00-12.13)	50.03 (24.86-251.43)	1.56	2.62	2.08	0.83
	60	2014	17.88 (11.31-29.20)	104.08 (51.21-905.06)	1.68	6.23	1.00	1.73

F.R. = Field recommended dose RR= Resistance Ratio RC = Resistance Coefficient RT= Relative toxicity

Table 3: Monitoring of resistance to tested biocides against *Spodoptera littoralis* collected from Kafer El- Sheikh

Biocides	FR ppm	Seasons	LC_{50} ppm	LC_{95} ppm	Slope	RR	RT	
Dipel 2x	-	Lab- strain	25.27 (11.88-49.28)	90.31 (43.75-170.11)	1.37	---	-	----
	64	2012	118.98 (74.38-243.81)	759.43 (365.99-7106.68)	1.49	4.71	1.27	11.87
	64	2013	128.60 (71.33-220.49)	865.01 (352.08-23987.38)	1.68	5.09	1.1	13.52
	64	2014	131.30 (85.10-222.44)	962.36 (423.33-19760.36)	1.47	5.19	1.00	15.04
Dipel DF	-	Lab- strain	27.46 (15.24-45.64)	110.51 (69.32-290.75)	1.27	--	-	---
	64	2012	67.88 (48.86-94.30)	337.63 (198.74-1082.12)	1.84	2.47	3.29	5.28
	64	2013	124.43 (108.35-124.16)	839.59 (394.81-6181.42)	1.55	4.53	1.33	13.12
	64	2014	141.84 (96.01-237.84)	1113.11 (493.99-11305.79)	1.43	5.17	1.00	17.39
Agerine	-	Lab- strain	48.55 (27.11-122.77)	197.33 (145.34-672.12)	1.01	---	-	---
	81.25	2012	126.38 (80.92-255.77)	838.34 (396.87-8321.32)	1.55	2.60	1.40	10.32
	81.25	2013	146.81 (96.92-258.99)	852.72 (358.29-17847.17)	1.69	3.02	1.38	10.50
	81.25	2014	170.99 (107.25-325.72)	1176.83 (503.77-23228.85)	1.53	3.52	1.00	14.48
Protecto	-	Lab- strain	24.68 (9.10-99.15)	187.33 (146.22-2525.07)	1.22	---	-	---
	141	2012	82.09 (50.35-144.02)	578.95 (303.13-2525.07)	1.32	3.33	2.03	4.11
	141	2013	85.62 (54.39-130.82)	773.36 (327.75-7752.24)	1.54	3.47	1.52	5.48
	141	2014	147.82 (92.62-240.29)	1176.19 (555.80-4766.04)	1.42	5.99	1.00	8.34
Radical	-	Lab- strain	0.44 (0.17-0.82)	3.63 (1.59-10.79)	1.66	---	-	---
	5	2012	2.05 (1.33-3.47)	11.81 (5.71-107.79)	1.69	4.66	1.72	2.36
	5	2013	2.25 (1.17-3.69)	16.56 (7.57-284.49	1.48	5.11	1.23	3.31
	5	2014	2.36 (1.45-3.92)	20.36 (9.25-153.64)	1.37	5.36	1.00	4.07
Radiant	-	Lab- strain	12.81 (10.24-14.23)	73.45 (41.35-360.89)	1.91	---	-	---
	30	2012	74.36 (54.71-105.94)	422.03 (262.46-1068.15)	1.58	5.80	1.57	14.07
	30	2013	84.85 (64.27-112.04)	481.07 (258.28-1899.88)	1.50	6.62	1.38	16.04
	30	2014	92.97 (67.89-145.42)	661.48 (321.14-3598.80)	1.84	7.27	1.00	22.05
Spintor	-	Lab- strain	2.87 (0.99-6.79)	14.65 (8.17-54.43)	1.39	---	-	---
	60	2012	5.24 (3.43-7.72)	30.41 (17.11-103.52)	1.68	1.83	3.28	0.51
	60	2013	17.99 (12.16-26.64)	91.62 (49.42-473.34)	1.90	6.27	1.09	1.53
	60	2014	19.39 (13.19-30.25)	99.62 (56.03-333.92)	1.72	6.76	1.00	1.66

F.R. = Field recommended dose   RR= Resistance Ratio   RC = Resistance Coefficient   RT= Relative toxicity

The results indicate that resistance ratio (RR) fluctuated from one year to another and from Governorate to another. During three cotton seasons (2012, 2013 and 2014), the tested *Bt* (Dipel 2x, Dipel DF, Agerine and Protecto showed low levels of resistance in all strains. The resistance ratios in Behera reached (5.86, 4.12, 1.83 and 4.99 fold) to Dipel 2x, Dipel DF, Agerine and Protecto) in cotton season 2014 (Table1). While RR reached (3.17, 4.94, 2.37 and 8.17 folds) in Gharbya and (5.19, 5.17, 3.52 and 5.99 fold) in Kafr El- Sheikh to the same *Bt*. Biocides respectively during cotton season 2014 (Tables 2 and 3).

Also, the same results for RR were obtained to Radical, Radiant and Spintor. It reached (4.25, 6.62 and 3.99 folds) in Behera, (3.43, 5.20 and 6.23 folds) in Gharbya and 5.36, 7.27 and 6.76 fold) in Kafr El- Sheikh respectively in cotton season 2014 (Tables 1, 2 and 3).

Sondoz *et al.* (2000) reported that the newly hatched larvae of *S. littoralis* were most sensitive to *Bt* toxin.

Abd- El- Hai (2001) evaluated the insecticidal activity as well as the latent effect of some *Bt* against the cotton leaf worm *S. littoralis* under field condition at Giza Governorate. The tested compounds were sprayed on cotton plants at the recommended rates using tap and saft El-laban artesian well water for dilution. The biological activity of *Bt* against cotton leafworm was enhanced with increasing the water pH.

The toxicity of the biocides (RT) at LC_{95} was decreased from season to another. For example, Dipel 2x was decreased by (1.98 and 2.31), (1.34 and 3.85)and (1.1 and 1.27) when it used in the seasons 2013 and 2014 in Behera, Gharbya and Kafr El- Sheikh Governorates respectively (Tables 1,2 and 3).

When resistance of the field strains were measured by resistance coefficient values (RC) to the tested biocides, results showed that the *Bt* compounds recorded high levels of resistance in all Governorates during 2014 cotton season of which reached (13.93, 9.23 and 15.04), (9.24, 10.55 and 17.39), (6.01, 8.12 and 14.48) and (6.95, 7.08 and 8.34) to Dipel 2x, Dipel DF, Agerine and Protecto in Behera, Gharbya and Kafr El-Sheikh respectively (Tables 1,2 and 3).

Also, the same results for RC were obtained with Radiant. RC for this compound reached (14.07, 11.58 and 22.05) in Behera, Gharbya and Kafr El-Sheikh. While Radical recorded medium levels of resistance (2.16, 2.29 and 4.07) and Spintor had low levels of resistance (1.21, 1.73 and 1.66) to the same field strains during cotton season 2014 (Tables 1, 2 and 3).

It's been noticeably found that the season to season decrease percentage of the biocides efficiency is to be followed by an equal increase percentage of Resistance Coefficient (RC).

The development of insect resistance is a very serious worldwide problem with > 400 species of insects and mites now resistant to one or more pesticides, including *Bt* sprays and *Bt* plants (Shelton *et al*, 2000).

Spinosad, the first member of the naturally is classified as a reduced risk insecticide and has been embraced by integrated pest management (IPM) practitioners as abiorational pesticides **(**Williams *et al*., 2003).

Ishaoya *et al.,* (2002) evaluated potency of emamectin benzoate (Radical), a novel insecticide for controlling S. littoralis. They found that a spray concentration on 25 mg AI / Liter in a cotton field resulted in over 90% suppression of *S. littoralis* larvae for 3 days only.

In conclusion, the biocides Radical and Spintor were very effective in the control of *S. littoralis* and they recorded lack and low levels of resistance after three cotton seasons. Therefore, in order to maximize the negative effects of the chemicals on the environment and natural enemies in the management of pests, the natural insecticides could be integrated into IPM programmers.

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