EVALUATION OF THE RESIDUAL EFFECTS OF THE MICROBIAL INSECTICIDE, *BACILLUS THURINGIENSIS SUBSPP.* ALONE OR MIXED WITH *PHTHORIMAEA OPERCULELLA* GRANULOSIS VIRUS (POGV) AGAINST *PECTINOPHORA GOSSYPIELLA* (LEPIDOPTERA: GELECHIIDAE)

By **KARIMA A. EL-LEBODY[1] AND ATEF S. ABD EL-RAZEK[2]**

[1] *Plant Protection Research Institute, Agricultural Research Center, Dokki , Giza , Egypt.*

[2] *Dept. of Pests & Plant Protection, National Research Centre, Dokki, Cairo, 12622, Egypt.*

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INTRODUCTION

Persistence of *Bacillus thuringiensis* on foliage is dependent on many environmental factors. Leong *et al.* (1980) concluded that sunlight exposure, leaf temperature and vapor pressure deficit contribute most to endospore decay. Sunlight, particularly ultraviolet radiation inactivates 50 % of *B. thuringiensis* spores within 30 minutes and 80 % within 60 minutes (Krieg, 1975). The inactivation of both spores and crystals is believed to be due to the production of peroxide or peroxide radicals following UV irradiation of the amino acids (Ignoffo and Garcia, 1978). Spores in absence of moist rapidly decay by exposure to sunlight and thus are very susceptible in very dry conditions (Pinnock *et al.*, 1971).

The insecticidal activities of *B. thuringiensis* against the pink bollworm *Pectinophora gossypiella* and other cotton pests have been widely investigated by Salama *et al.* (1983a) and El-Lebody *et al.* (2003).

Also, *P. gossypiella* is known as an alternative host to certain lepidopterous entomoviruses, e.g., *P. gossypiella* is an alternative host to the *Autographa californica* nuclear polyhedrosis virus (AcNPV). In this regard, Ismail (2000) found that the newly genetically modified baculovirus (AcMNPV) is 2.5 X more efficient than the wiled type (AcNPV) against 2nd instar larvae of *P. gossypiella.*

Few studies on field trials involving combinations of NPVs or Granulosis viruses (GV) with *B. thuringiensis* have been reported. In this regard, Takchev (1987), found that, the mixture of Bitoxibacllin (*B.t.subsp. thuringiensis*) at 0.25%

and a virus preparation (virin GYaP) at $4.5x10^8$granules/liter, representing half their normal optimum concentrations, gave technical effectiveness 70% against *Cydonia pomonella* on apple. While, levels of technical effectiveness between about 62% & 74% were obtained when the Bitoxibacllin was applied at the normal rate of (2.5 Kg / ha) with virin at half the normal rate (150 ml / ha).Also, Moawad *et al.* (1998) indicated that, the application of both *B.t.* ($32x10^6$IU/g) and Gv($5X10^9$granules/g) mixture gave much better results than both of them separately against potato tuber moth, *Phthorimaea operculella* in potato cultivations.

The present study aims to cover three purposes:

1- Evaluation of the efficacy of *B. t. kurstaki*, *B.t.aizawai* and *P. operculella* granulosis virus (PoGV) and their mixtures against pink bollworm (PBW).

2- Evaluation of residual spore count of *B. t. kurstaki*, *B.t.aizawai* either alone or mixed with PoGV at different periods after application on cotton plant bolls.

3- Evaluation of the former residual spore count of *B. t. kurstaki*, *B.t.aizawai* efficacy against the 1*st* instar larvae of PBW.

MATERIAL AND METHODS

I- The field experiments:

Field experiments were carried out in an area of 1/3 Fadden cultivated with the cotton variety "Giza-85" during the two successive seasons 2001 / 2002 & 2002 / 2003 in Sharkia Governorate to evaluate the efficacy of some bio-insecticides and their binary mixtures against the pink bollworm (PBW).

The experimental area was divided into 7 treatments splited to 3 replicates each and each replicate about 42 m^2.Tested microbial insecticides were applied by knapsack motor sprayer at 400 L /Fadden, using complete randomize design.

Each treatment was sprayed 3 times during the two experimental seasons of 2002 & 2003 (late plantation) on cotton plants, weekly at 3rd Sept., 10^{t}h Sept. & 17th Sept. during2002;and 12th Sept., 19th Sept. & 26th Sept. during 2003.Green boll samples (25 boll each) were collected randomly before spray and at 7 days post spray from all treated and untreated areas. These samples were examined and dissected in

the laboratory, to estimate the total number of PBW larvae/treatment and subsequently, the percentage of both small and full grown larvae were determined.

TABLE (I)

Microbial insecticides used in the study and their commercial names and recommended doses.

Microbial insecticide	Commercial name	Recommended rate (g/Feddan)	source
B. thuringiensis var. aizawai	XenTari	500	Bayer
B. thuringiensis var. kurstaki	Dipel-2X	300	Abbot-Laboratory
Phthorimaea operculella granulosis virus (PoGV)	Virotecto	300	Insect pathogen unit PPRI , ARC
PoGV	Virotecto	150	Insect pathogen unit PPRI , ARC
B.t. var. aizawai + PoGV	XenTari + poGV	500+150	
B.t. var. kurstaki + PoGV	Dipel-2X + poGV	300+150	

Efficacy of tested compound was based on total larval dip counts after each spray and reduction of infested bolls with PBW. Reductions of Pink bollworm infestations were calculated according to Henderson and Tilton equation (1955).

Semi-field experiments were carried out to determine the residual of *B.t.* subspp. Spores at recommended rates either alone and/or in combination with *P. operculella* granulosis virus (PoGV) at 150 g / fed. For this purpose, the cotton variety "Giza-85" was planted in pots (30 cm in diameter) and left in open air in Sharkia governorate during cotton season 2004. The formulations of each bio-insecticide were suspended in 1/2 L water and sprayed by pressure sprayer (1.1 Litter) on cotton plants, of age 5 months, (15 pots/treatment) at 6-7 p.m. After an one hour, one day, three days and seven days post spray, randomize 3 cotton bolls/treatment were taken with care to avoid contact, and placed individually in polyethylene bags and kept under cooling condition till transportation to the laboratory.

Isolation of residual *B. thuringiensis* subspp. from cotton plants:

Residual spray deposits on cotton bolls were assessed following the techniques of Salama *et al.* (1983b) as follow:

An area equivalent to 1 cm^2 was cut with a sterile cork borer from one side of the boll surface. These discs were transferred to sterile test tubes filled with 100 ml sterile distilled water. The tubes were shaken for 20 min., Drop of these isolated bacterial strains from surface of bolls were confirmed to be the originally sprayed strains by phase contrast and the count of spores in 1 ml water was recorded after pasteurization of the solution at 80°C for 3 sec. Then a dilution of the solution was done to 1×10^{-2} and 1×10^{-4}, and a nutrient agar plates were prepared and a drop from each dilution was streaked on the plate. The sporulation (number of bacterial spores/ml of harvest), and the endotoxin yield (g/L) were determined according to Abdel-Razek (1995).

Laboratory experiment:

The activity of the residual spore count of *B.t.* subspp. at different periods after application on cotton bolls (either isolated or produced from isolated strains) were evaluated against the 1st instar larvae of PBW as follows : a volume of 1 ml suspension of each treatment at each different periods after application was homogenated and mixed with 1 gm of artificial diet in Petri dish (9 cm in diameter), forty newly hatched larvae of PBW (Lab. strain) were placed on the surface of the diet, in addition Petri dishes were prepared with the same diet, but only mixed with water and used as control and 40 neonate PBW larvae were placed on the surface of diet. Larvae were allowed to feed on the treated diet for 1½ hour, then transferred individually to glass tubes (2 x 7 cm) containing untreated artificial diet. All tubes were plugged with absorbent cotton and incubated at 26±1°C and 80±5 % R.H.

The larval mortality/treatment was recorded and corrected by Abbott's formula (1925). Also, decrease in activity of recorded mortality was calculated by the formula:

The decrease in activity =

(%mortality in control-%mortality in treatment)/%mortality in control)*100

RESULTS AND DISCUSSION

Efficacy (%) of *B. t.* formulations against PBW larval population:

Data in Tables (2 & 3) illustrate that the PBW small larvae (%) and PBW full-grown larvae (%) before spraying with Xentari were (0.0 % & 71.4 %) and (36.7 % & 6.6 %) during cotton season 2002 and cotton season 2003, respectively. It is very clear that the PBW small larvae (%) before spraying during cotton season

2002 decreased than it during cotton season 2003. Meanwhile, the PBW full-grown larvae (%) of the total larval population increase before spraying during cotton season 2002, than in cotton season 2003. So, the efficacy (%) of XenTari during cotton season 2002 was higher than it during cotton season 2003.The efficacy of XenTari against PBW larval population during cotton season 2002 was 93 %, 94 % & 82 % after 1^{st}, 2^{nd} & 3^{rd} spray, respectively. During cotton season 2003, the corresponding efficacy was 40 %, 69 % & 36 %, respectively. This result agrees with Lesser (1987) who indicated that, the efficacy of the products based on *B.t.* subsp. *israelensis* against mosquito was found to be variable and dependent upon larval age and density, they were most effective against low to moderate population of small larvae. Also, this result becomes clearer with the efficacy (%) of Dipel-2X against PBW larval population and so on for all treatments. However, data in Tables (2 & 3) show that, the PBW small larvae and PBW full-grown larvae (%) before spraying Dipel-2X were (18.2 % & 63.6 %) and (90.0 % & 0.0 %) during cotton seasons 2002 & 2003, respectively. So, during cotton season 2002, the efficacy (%) of Dipel-2X was 97 %, 98 % & 91 % after 1*st*, 2*nd* & 3*rd* spray, respectively. During cotton season 2003, the corresponding efficacy (%) was 10 %, 45 % & 37 %, respectively (Tables 2 & 3).

The efficacy (%) of *Phthorimaea operculella* granulosis virus (PoGV) at recommended and ½ recommended rate against PBW larval population:

Data in Tables (2 & 3) indicate that the PBW small larvae and PBW full-grown larvae (%) before spraying of PoGV (at the recommended rate) were 0.0 % & 100 % and 50 % & 0.0 % during cotton season 20002 & 2003, respectively. The efficacy (%) after 1*st*, 2*nd* & 3*rd* spray were 92 %, 18 %, 93 %, -690 % & 65 %, -897 % during cotton season 2002 and 2003, respectively. Also, PoGV have high efficacy (%) when used at ½ recommended rate against PBW larval population during cotton season 2002. However, data in Tables (2 & 3) show that, the PBW small larvae and PBW full-grown larvae (%) before spraying of PoGV (½ recommended rate) were 16.7 % & 66.6 % and 62.5 % & 12.5 % during cotton season 2002 and 2003, respectively; and the efficacy (%) after 1*st*, 2*nd* & 3*rd* spray were 93 %, -74 %, 86 %, -81 % and 62 %, -227 % during cotton season 2002 and 2003, respectively.

The efficacy (%) of *B.t.* subspp.-PoGV mixture against PBW larval population:

Data in Table (2) indicate that the both of binary mixture of Xentari (500 g/fed.) + PoGV (150 g/fed.) and the binary mixture of Dipel-2X (300 g/fed.) + PoGV (150 g/fed.) have high bio-efficacy (%) ranged between 89 % & 98 %

during cotton season 2002. While, the efficacy (%) of these mixtures ranged between 1.0 % & -62 % during cotton season 2003. During cotton season 2002, the PBW small larvae and PBW full-grown larvae % before spraying with Xentari PoGV mixture and Dipel-2X - PoGV mixture were 39.9 %, 55.5 % & 0.0 %, 84.6 %, respectively. During 2003, the corresponding percentages were 47.3 %, 26.3 % & 45.5 %, 27.3 %, respectively (Tables 2 & 3).

TABLE (II)

The pink bollworm (PBW) small and full-grown larvae (%) before spraying, efficacy (%) of treatments against both of PBW larval population after each spray and PBW Infestation after 3 sprays during cotton season 2002.

Treatments	PBW larvae % before spraying		Efficacy (%) of treatments after each spray			% Boll infestation	% efficacy of treatments
	Small	Full-grown	1st	2nd	3rd	Before spray	after 3 sprays
XenTari (500 g/fed.)	0.0	71.4	93	94	82	16.0	84.5
Dipel-2X (300 g/fed.)	18.2	63.6	97	98	91	13.3	92.5
PoGV (300 g/fed.)	0.0	100.0	92	93	65	17.3	81.8
PoGV (150 g/fed.)	16.7	66.6	93	86	62	14.6	74.6
XenTari + PoGV (150 g/fed.)	39.9	55.5	98	95	89	18.7	89.1
Dipel-2X + PoGV (150 g/fed.)	0.0	84.6	98	98	96	14.6	94.9

The efficacy (%) of *B.t.* formulations, *P. operculella* granulosis virus (PoGV) and their combinations against PBW infestation:

Data in Tables (2 & 3) illustrate the infested bolls (%) before spraying and the efficacy (%) of treatments against PBW infestation. However, during cotton season 2002, the efficacy (%) of both XenTari and XenTari - PoGV mixture against PBW infestation were 84.5 % & 89.1 %, respectively. During cotton season 2003, the corresponding efficacies (%) were 16.6 % & -97.0 %, respectively (Tables 2 & 3). During cotton season 2002 and 2003, the efficacy (%) of Dipel-2X against PBW infestation was 92.5 % & -125.1, respectively. The corresponding efficacy (%) of

Dipel-2X - PoGV mixture was 94.9 % & -50.1 %, respectively (Tables 2 & 3). The efficacy (%) of PoGV (300 g/fed.) against PBW infestation was 81.8 % and -557.0 % during cotton season 2002 and 2003, respectively. The corresponding efficacy (%) of PoGV (150 g/fed.) was 74.6 % & -147.3 %, respectively (Tables 2 & 3). As reported by El-Lebody (2003), the differences in each treatment efficacy (%) from season to another is due to the relation between the used insecticide efficacy and both of PBW small larvae (%) and the ages of the PBW larvae inside the infested bolls before spraying. And based on this relation, she invited a primary table to help in determining the proper time for starting of spray, kind and rate of the used insecticide against PBW infestation. Also, the present study indicated that, Xentari, Dipel-2X and their binary mixtures with PoGV (at½ rate) were shown to be more efficient against PBW infestation than PoGV at used rate or at ½ rate. But, because of this result record of the primary study on the PoGV in combination with *B.t.* formulations, caution should be exercised in proceeding with such combinations for control of PBW in the cotton fields without adequate field testing.

TABLE (III)

The pink bollworm (PBW) small and full-grown larvae (%) before spraying, efficacy (%) of treatment against both of PBW larval population after each spray and PBW infestation after 3 sprays during cotton season 2003.

Treatments	PBW larvae % before spraying		Efficacy (%) of treatments against PBW Larval population after each spray			% Boll infestation	% efficacy of treatments
	Small	Full-grown	1*st*	2*nd*	3*rd*	Before spray	after 3 sprays
Xentari (500 g/fed.)	36.7	6.6	40	69	36	26.6	16.6
Dipel-2X (300 g/fed.)	90.0	0.0	10	45	-37	8.0	-125.1
PoGV (300 g/fed.)	50.0	0.0	18	-690	-897	2.66	-577.0
PoGV (150 g/fed.)	62.5	12.5	-74	-81	-227	9.3	-147.3
Xentari + PoGV (150 g/fed.)	47.3	26.3	-42	0.5	-28	17.3	-97.0
Dipel-2X + PoGV (150 g/fed.)	45.5	27.3	-6.4	1.0	-62	12	-50.1

We can conclude from data in Tables (2 & 3) that, the efficacy (%) of each treatment during cotton season 2002 was higher than during cotton season 2003: this resulting due to decrease of PBW small larvae (%) with increase of PBW full-grown larvae (%) of the total larval population before spraying with each treatment. These results are very logic because of the PBW biology and feeding behavior. Also, Abd El-Salam *et al.* (1991) reported that, the young larvae in relation to the total number of the larvae counted in the infested bolls seemed to be more reliable parameter for starting the control program and it was found to be helpful criteria for evaluating bollworms control programs. Also, these results are in great agreement with those reported by El-Lebody *et al.* (2001).

Semi-field experiments:

To determine residual distribution of *B. thuringiensis* strains used either alone or in combination with PoGV after spraying the cotton bolls, samples were collected at 0, 1, 3, 7 days after spraying cotton in the season, 2004. Data in Table (4) showed, generally, an obvious decrease in *B.t.* viable spore counts after 7 days post spraying of bolls. Also, the decrease in spore count was less in case of spraying the *B.t.* strains in combination with the (PoGV). So, we could conclude that the survival of both *B.t.* subsp. *aizawai* and *B.t.* subsp. *kurstaki* was affected by the time it remains on the cotton bolls in the semi-field tests. To determine the ability of residual *B.t.* subspp. used to infect the pink bollworm, a mortality was made longer residual activities (10 days) reported some viable endospores of *B. thuringiensis* subsp. *kurstaki* have been recovered from foliage one year after ground application of *B. thuringiensis* subsp. *kurstaki* (1 billion international units (BIU)/tree) (Reardon and Haissig, 1984). Formulations of *B.t.* remain biologically active on foliage for only a brief period, with half-life estimates ranging from one to two days on unwashed foliage (Ignoffo *et al.*, 1974; Morris, 1983; Pozsgay et al. 1987 and Pusztai et al. 1991) and from 20 to 30 days on shaded foliage (Beckwith and Stelzer, 1987). In general, *B. thuringiensis* losses 50 % of its insecticidal activity in field within 1-3 days, often necessitating a second spray application for control of insects, such as the gypsy moth (*Lymantria dispar* Linnaeus), spruce budworm (*Christoneura fumiferana* Clemens) (McLeod *et al.*, 1983). The data reported in Table (5), showed a decrease in spore count reported after the different periods of investigation and subsequently, decrease in percent mortality of 1*st* instar larvae of *P. gossypiella* as the time increase post spraying.

Table (5) showed a 33.3 % decrease in activity of *B.t.* subsp. *aizawai* against 1*st* instar of *P. gossypiella* after one day of spraying the cotton bolls. Also, a

100 % decrease in activity of *B.t.*subsp.*aizawai* recorded after 7 days post spraying. In case of combination with (PoGV), the activity against the pink bollworm at the zero time (control) was high for *B.t.* subsp. *aizawai* compared with its application alone due to the combined effect of bacteria and virus against the larvae. One day after spraying, a 39 % decrease in activity was recorded while a 86.8% decrease in activity recorded 7 days post-spraying the combination on the bolls. On the other hand, a decrease of 14.3 and 27.0 % in activity of *B.t.* subsp. *kurstaki* alone against *P. gossypiella* 1st instar larvae 1 and 3 days post spraying, respectively. Studies with the activity in combination with (PoGV) showed a decrease of 34 and 51 % in activity of *B.t.* subsp. *kurstaki* against 1*st* instar larvae of *P. gossypiella* 1 and 3 days post spraying (Table, 5).

TABLE (IV)

Residual spore count of B. thuringiensis varieties alone or in combination with (PoGV) at different periods after application on cotton plant bolls in Sharkia governorate undersemi-field condition.

Treatments	No. of *B.t.* spores/cm² boll disc after application			
	0 (day)	1 (day)	3 (days)	7 (days)
B. thuringiensis var. aizawai	15×10^5	11×10^5	10×10^5	45×10^4
B. t. var. aizawai + (PoGV)	14×10^5	84×10^4	56×10^4	27×10^4
B. thuringiensis var. kurstaki	23×10^5	15×10^5	12×10^5	34×10^4
B. t. var. kurstaki + (PoGV)	19×10^5	12×10^5	85×10^4	24×10^4

Table (V)

Comparative studies on activity of residual spores of *B. thuringiensis* varieties alone or in combination with (PoGV) at different periods after application against 1st instar larvae of *P. gossypiella*.

Treatments	% Mortality of 1st instar *P. gossypiella* after different periods (/days) and decrease in activity(d.a.)						
	0	1	(d.a.)	3	(d.a.)	7	(d.a.)
B. t. aizawai	32.1	21.4	33.3	-	-	0.0	100
B. t. aizawai+ (PoGV)	67.9	41.4	39.0	-	-	11.8	86.8
B. t. kurstaki	88.0	75.4	14.3	64.2	27.0	-	-
B.t. Kurstaki + (PoGV)	69.2	46.0	34.0	34.0	51.0	-	-

Laboratory experiments:

Data in Table (6) showed a gradual decrease in mean number of spore count of *B.t.* subsp. *aizawai* either alone or in combination with PoGV after the different periods post spraying. The endotoxin yield of *B.t.* subsp. *aizawai* sprayed

alone showed also, an obvious decrease at all the period tested compared with the control ones (0 day), but the endotoxin yield showed an obvious decrease at the 3 and 7 days, (Table, 6).

The percent mortality of neonate larvae of *P. gossypiella* as affected by *B.t.* subsp. *aizawai* produced from isolated residual spores, sprayed alone on cotton bolls, showed gradual decrease in mortality by 3, 16 and 45 % at 1, 3 and 7 days, respectively, as compared with that observed for control at (0) day, (Table, 6).

On the other hand, the decrease in mortality of neonate *P. gossypiella* as affected by *B.t.* subsp. *aizawai* produced from isolated residual spores sprayed in combination with (PoGV) showed a 15 and 32 % decrease at 1 and 7 days post spraying, respectively, as compared with the control, (Table, 6).

Table (7) showed the same trend in sporulation and endotoxin yield when cotton bolls were sprayed by *B.t.* subsp. *kurstaki* either alone or in combination with (PoGV). In mortality studies, decreases in activity against P. gossypiella reached 12 and 28 % at 1 and 7 days, respectively, post spraying of the bacterial spores alone compared to the control. While, a 19, 26 and 27 % decrease were recorded at 1, 3 and 7 days, respectively, compared with the control ones of bacterial spores sprayed in combination with (PoGV), (Table, 7).

TABLE (VI)

Efficacy of *B. thuringiensis var. aizawai* produced under laboratory conditions from isolate dresidual bacterial spores after different periods of spraying alone or in combination with (PoGV) on cotton plants.

Parameters	*B .t. var. aizawai* Isolated from different Periods (days)				*B.t. var. aizawai*+ PoGV Isolated from different Periods (days)			
	0	**1**	**3**	**7**	**0**	**1**	**3**	**7**
Sporulation (spore/ml)	8.4 x 10^{10}	7.6 x 10^{10}	6.6 x 10^{10}	5 x 10^{10}	7.8 x 10^{10}	7.6 x 10^{10}	7 .0 x 10^{10}	6.8 x 10^{10}
Endotoxin yield (g/L)	9.15	7.2	7.2	7.5	9.00	8.1	7.5	7.00
% Mortality against neonates Of PBW	80.5	78.1	67.3	44.5	75.5	64.1	-	51.0
Decrease in activity		3 %	16.4	45		15 %		32 %

From the previous results, it could be concluded that the residual spores of *B.t.* subspp. is consistently correlated with different environmental factors attained on cotton plants.

The little gradual decrease in viable spore count, isolated from cotton bolls from 0-7 days, may be attributed to the fact that both Xentari and Dipel-2X are commercial products that may contain some protectants that lead to its long persistence.

TABLE (VII)

Efficacy of *B. thuringiensis* var. *kurstaki* produced under laboratory conditions from isolated residual bacterial spores after different periods of spraying alone or in combination with (PoGV) on cotton plants.

Parameters	*B.t var.Kurstaki* Isolated from different Periods (days)				*B.t var.Kurstaki* +PoGV Isolated from different Periods (days)			
	0	1	3	7	0	1	3	7
Sporulation (spore/ml)	9.1 x 10^{10}	8.6 x 10^{10}	7.4 x 10^{10}	7.4 x 10^{10}	8.2 x 10^{10}	7.8 x 10^{10}	7.1 x 10^{10}	6.0 x 10^{10}
Endotoxin yield (g/L)	9.65	8.1	8.00	8.00	8.5	7.5	8.1	8.1
% Mortality Against neonates of PBW	73.6	64.8	-	53.3	74.3	60.0	55.0	54.3
Decrease in activity		12%		28%		19%	26%	27%

SUMMARY

Residual activity of sprayed spores of *B.t. kurstaki* and *B.t.aizawai* showed a gradual decrease in spore count at all treatments till the seventh day post spraying. The decrease in spore count of treatments conjugated with *Phthorimaea operculella* granulosis virus (PoGV) was more obvious in both the strains tested. Also, a decrease in activity against pink boll_worm (PBW)*Pectinophora gossypiella* 1^{st} instar larvae was recorded in all periods as compared to the control (0-day).

Data on spore counts of produced *B.t.* subspp. in the lab. was generally decreased after 3 and 7 days post-spraying, also with a gradual decrease in PBW neonate larval mortality at all periods of testing (1, 3,&7 days) as compared to 0 day.

Field application with two formulations of *B.t.*, PoGV and their binary mixture are effective on its own against pink bollworm (PBW) larval population and PBW infestation of cotton bolls. But, they were not reliably effective when the PBW small larval population (%) increase and PBW full-grown larval population (%) decrease before spraying.

Key words: *B. t.* formulation, *Phthorimae operculella* granulosis virus (PoGV) *Pectinophora gossypiella* , residual studies

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