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ACCELERATING INFESTATION OF PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) (LEPIDOPTERA: GELECHIIDAE) ON BT–VARIETIES OF COTTON IN PAKISTAN

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ABSTRACT

Bt cultivars of cotton worked satisfactorily for last many years except some limited and short duration problems of bollworms reported from different pockets, that were usually because of mixed seed. In last cotton season 2014, pink boll worm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) intensified cotton crop tremendously and it was reported from almost all the cotton growing tract of Punjab. Owing to these reports, present studies were carried out in three subdivisions/tehsils of Lodhran district (Kehror Pacca , Dunya Pur and Lodhran) on 17 different Bt-varieties/lines of cotton (A-777, BS-52, Bt-312, Bt-252, Bt-616, Eagle-1, IUB-2013, MNH-992, NIAB-999, SG-1, A-555, CEMB-666, FH-142, IUB-222, Lalazar, MNH-886 and MNH-988). Fifty bolls were plucked randomly from all the fields. The varying number of samples of cotton bolls (50 bolls from each variety) were collected for consecutive 15 days (20th October to 5th November, 2014) from different cotton cultivars grown at farmer's fields in Kehror Pacca (11 samples), Dunya Pur (10 samples) and Lodhran (9 samples) sub-divisions. In total 1549 collected bolls were kept in paper bags for a week and then percentage of damaged bolls as well as number of live larvae were recorded from all samples. The results were alarming to note that all of 17 cultivars/lines were susceptible showing maximum infestation of 24.49% to 100%. The average intensity of alive larvae per boll was 109.6 % (1698 larvae in 1449 bolls collected for study) inside the infested bolls. The situation spells a serious threat and demands the immediate attention of cotton growers, breeders, biotechnologists, seed producers, extension workers, entomologists and research workers.

Keywords: Bollworm, Infestation, *Pectinophora gossypiella*, Transgenic cotton

INTRODUCTION

Pakistan is the fourth largest producer of cotton (*Gossypium hirsutum* L.) in the world after China, USA and India. It is the most important cash crop, cultivated on 2.879 million hectares in our country. It is not only source of large amount of foreign exchange contributing about 7.0% of value added in agriculture and about 1.5% of GDP but also contributes about 66.50% share in national oil production (Economic Survey of Pakistan, 2013). It is also a reasonable source of income for poor unskilled village labor and farming community, particularly women. The rural population constitutes more than two third of total national population (Zeeshan *et al*., 2010).

Cotton is victim of a complex of insect pests including sucking pests and bollworms all over the world (Abdullah, 2010), but since the introduction and adoption of Bt cotton on larger scale in Pakistan, bollworm complex especially *Helicoverpa armegera* Hubner (Lepidoptera: Noctuidae) pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) and spotted bollworms, *Earias insulana* Boisd as well as *Earias vitella* Fab. (Lepidoptera: Arctidae) are not a major problem in Bt cotton (Dhillon *et al*., 2011). The pink bollworm, *P. gossypiella* (Saunders) is considered to be one of the most injurious cotton pests because it is difficult to control with insecticides (Lykouressis *et al*., 2005). The pink bollworm larvae feed on flower buds, flowers, bolls, developing seeds within bolls, damage seeds

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and destroy the lint. The termination of boll growth results in boll rotting, premature or partial boll opening, deterioration of lint quality and reduction of staple length and strength. It increases quantity of trash content in the lint and impurities in cotton seed oil and oil cake.

Introduction of genetically modified (GM) crops had dramatic adaptability, not only in technologically advanced countries but also in developing countries and consequently area under GM crops increased 100 times i.e., 1.7 million hectares in 1996 to 170 million hectares in 2012. The performance of GM cotton cultivars in the field and encouraging reports from experts and researchers increased the adoptability ratio in farmers and Pakistan has become fourth Bt cotton grower of the world by utilizing its indigenous cotton varieties to transgenic one through genetic modifications (Spielman *et al*., 2014). A significant change in cropping scheme in the cotton growing areas has been witnessed with the introduction of Bt varieties of cotton in Pakistan (PWQCP, 2014). Bt varieties, supposed to have resistance against *H. armigera* while other bollworms, have gained a very rapid adoption among farming communities, and now Bt varieties have almost replaced the conventional non Bt varieties in district Lodhran in particular and in Pakistan in general. By the introduction of Bt. Varieties, the bollworm problem has been solved to some extent except armyworm, but the problem of sucking insect pests attack has remained unsolved still now (Ahsan and Altaf, 2009; Abdullah, 2010, PWQCP, 2014)

The present study was carried out in Lodhran to determine the extent of infestation and damage of pink bollworms in cotton varieties sown in farmer fields of three sub-divisions of Lodhran district.

MATERIALS AND METHODS

The experiment was conducted in three different subdivisions of district Lodhran (Kehror Pacca , Dunya Pur and Lodhran), a fertile cotton growing area of southern Punjab, Pakistan (29.40 latitude and 71.68 longitude (Maps, 2014) near Sutluj river during the cotton season 2014. The varying number of samples were collected for consecutive 15 days (20th October to 5th November, 2014) from different cotton cultivars grown at farmer's fields at Kehror Pacca (11 samples), Dunya Pur (10 samples) and Lodhran (9 samples) sub-divisions to observe infestation and damage of pink bollworm. The seeds of the cultivars were arranged by the farmers from market or from the breeders or their close farmers. It may be added that in some cases the sources of seed were not authenticated and the name given to the line was hidden and in few cases fictitious. Out of seventeen tested cultivars, ten cultivars viz., A-777, BS-52, Bt-312, Bt-252, Bt-616, Eagle-1, IUB-2013, MNH-992, NIAB-999 and SG-1 were candidate lines, whereas, 7 of them viz., A-555, CEMB-666, FH-142, IUB-222, Lalazar, MNH-886 and MNH-988 were the standard varieties approved for general sowing among the farmers.

The samples of nearly 50 unopened mature bolls were taken randomly from standing cotton crop in the sampling period. One sample from each variety was taken randomly from each subdivision /tehsil so that maximum number of cultivars could be examined. The cultivars were not standardized; rather random sampling was conducted from the pool of cultivars available to and cultivated by the farmers in selected locality. The sample of bolls collected from each variety was taken in a paper envelope and kept in the office lab for 10 days to let the larva be grown up if it is susceptible and die if it is resistant. The bags were opened and the collected bolls were dissected to observe the damage caused by the pink bollworm (PBW) and larvae feeding inside were located and counted. The larvae which were grown up when collected from field had transformed into a pupa. So the larvae successfully pupated on these Bt cultivars were also counted and recorded in the data as a parameter of the study.

Following parameters were calculated from the data collected from each tehsil/subdivision by formulae mentioned below.

$$\% \text{ infestation of PBW} = \frac{\text{Number of infested bolls}}{\text{Number of total bolls in the sample}} \times 100 \quad (1)$$

$$\% \text{ larval population per boll} = \frac{\text{Number of larvae in infested bolls}}{\text{Number of total infested bolls in the sample}} \times 100 \quad (2)$$

$$\% \text{ pupal population per boll} = \frac{\text{Number of pupae in infested bolls}}{\text{Number of total infested bolls in the sample}} \times 100 \quad (3)$$

$$\% \text{ larval field intensity per boll} \frac{\text{Number of larvae in infested bolls}}{\text{Number of total bolls in the sample}} \times 100 \quad (4)$$

$$\% \text{ pupal field intensity per boll} = \frac{\text{Number of pupae in infested bolls}}{\text{Number of total bolls in the sample}} \times 100 \quad (5)$$

The collected and computed data were analyzed by ANOVA technique using Statistix 8.1 software to observe the significance of the difference among various tehsils and cotton cultivars/line. The comparisons of means were made by Tuckey's HSD test at 5 % level of significance.

RESULTS

The results revealed that out of 1549 collected bolls, 1232 bolls (79.5 %) were infested yielding 1698 larvae (109.6 % of total bolls collected for test and 137.8% of the infested bolls in the sample). It was also observed that this infestation was 100% by only one bollworm Pink Bollworm *P. gossypiella*. No larva of *H. armegera* or spotted bollworms *E. insulana* and *E. vitella* were found in the samples.

A significant difference regarding %age infestation levels of PBW among tehsils/sub-divisions of district Lodhran was recorded ($p < 0,05$) (Table 1). Kehror Pacca and Dunya Pur had higher % age infestation of PBW as compared to tehsil Lodhran. Infestation %age of Kehror Pacca and Dunya Pur

was statistically indifferent, but % age infestation of subdivision Lodhran was different from both of the subdivisions Kehror Pacca and Duny pur (Table 1).

Average larval and pupal population of Pink Bollworm per boll in infested bolls did not show any statistical difference in all the subdivisions(Table 1). The %age average larval and pupal intensity in the field was higher in Kehror Pacca and Dunya Pur as compared to tehsil Lodhran (Table 1). The pupal density of Kehror Pacca was found statistically different from that of Lodhran but similar to Dunya Pur (Table 1).

Since it was a basic survey and so much infestation was unexpected, so the repetitions required in a statistical analysis were not available. Since the number of repeats in cultivar wise samples is not enough to represent a proper statistical comparison, so a non parametric comparison of all the cultivars studied has been shown in (Table 2). The results show that all the varieties/cultivars/lines did not exhibit resistance against pink bollworm demonstrating infestation in the range of 23.3-98.1%. The lowest infestation (23.3%) was recorded in BS-52 while the highest infestation (98.1%) was observed in Bt-312 (Table 2).

The minimum density of PBW larvae per boll (0.83 larva/boll) was recorded in MNH-988 while the maximum density of PBW larvae per boll was observed in cultivar A-555 (3.37 larvae/boll). It is evident from the results that live larvae recovered from bolls after10 days of the actual collection from the field were taking the feed from bolls without any lethal effect of Bt proteins on larval stage of the pest (Table 3). On an average, varieties exhibited 1.2 to 1.6 alive larva/infested boll from the bolls of the cotton taken as sample from the fields of standing Bt varieties in three subdivisions/tehsils of Lodhran district.

Table 1

ANOVA parameters and comparative features of infestation of pink bollworm in samples of bolls collected from different tehsils/subdivisions of district Lodhran in 2014.

Name of subdivision/tehsils sampled in district Lodhran	%age infestation of PBW in tested bolls	%age larval population per boll in infested bolls	%age Pupal population per boll in infested bolls	%age larval intensity in field out of total bolls	%agePupal intensity in field out of total bolls
Kehror pacca	94.4 A	156.6 A	11.1 A	146.2 A	10.6 A
Dunya Pur	87.4 A	113.1 A	9.1 A	99.6 AB	8.0 AB
Lodhran	49.5 B	151.1 A	10.1 A	75.4 B	5.2 B
Average of district	77.1	140.3	10.1	107.1	7.9
P values	0.00	0.84	0.63	0.004	0.038
F vaue	48.1	2.71	0.46	6.73	3.67

Table 2.

Infestation of PBW in boll-samples of different cotton varieties collected from three subdivisions of district Lodhran in year 2014.

Name of cultivar	No. of Samples	% infestation of pink bollworm		
		Maximum	Minimum	Average
A 555,	2	95.8	94.0	94.9
A 777	3	100.0	40.0	80.0
IUB 222	1	24.5	24.5	24.5
Bt 252	2	100.0	82.7	91.3
Bt 312	1	98.1	98.1	98.1
BS 52	2	88.0	55.0	71.5
Bt 616	2	90.0	79.0	84.5
CEMB 666	1	92.3	92.3	92.3
Eagle1	1	92.0	92.0	92.0
MNH 988	3	90.7	55.3	77.4
NIAB 999	1	78.0	78.0	78.0
SG1	1	70.2	70.2	70.2
FH 142	3	100.0	64.0	88.0
IUB 2013	1	50.0	50.0	50.0
Lalazar	3	100.0	45.1	81.0
MNH 886	2	84.0	65.4	74.7
MNH 992	1	40.4	40.4	40.4
Overall average	1.8	82.0	66.2	73.0

DISCUSSION

The results observed from the samples had unexpected results because at least from last 06 years the infestation of bollworms, including pink bollworm on cotton, has declined to almost 0 levels (Iqbal *et al.*, 2014; PWQCP, 2015). Iqbal *et al*. (2014) had conducted analysis of three cotton cultivars (FH 142, MNH886, FH Lalazar) and reported that all the three varieties had sufficient quantity of Bt toxin in leaves, mature stem, 15 and 30 days old bolls, fully matured bolls, roots and secondary roots. Bt toxin gradually decreased from top to down up to 8th leaf possessing 2.28 µg /g fresh weight which is sufficient to control Lepidopteron pests. No resistance in all bollworms against Bt toxin was reported for last few years. But this year, infestation of PBW to an unexpected extent was recorded in all of the tested varieties. In three samples of FH-142, 88% infestation (range 64 to100%); in three samples of Lalazar 81% infestation (range 45.1 to 100%) and in two samples of MNH-886, 74.7% infestation (range 65.4 to 84%) of PBW was recorded. Although the factor responsible for and circumstances promoting it are yet to be explored, even then, this situation has strong reasons to believe that PBW has gained the resistance against all Bt cultivars cultivated in Pakistan. Nevertheless, the same cultivars are still performing well against *Helicoverpa* spp. and *Earias* spp. in the same conditions.

Table 3

Intensity of PBW in infested bolls of the samples of different varieties collected from three subdivisions/tehsils of district Lodhran in year 2014.

Name of cultivar	Number of samples	No. of bolls examined	Number of bolls infected	Number of live larvae	Minimum density/infested boll	Maximum density/infested boll
A 555,	2	98	93	213	1.23	3.37
A 777	3	147	123	139	1.34	1.69
IUB 222	1	49	12	15	1.25	1.25
Bt 252	2	111	100	165	1.17	2.23
Bt 312	1	53	52	77	1.48	1.48
BS 52	2	90	66	83	1.07	1.64
Bt 616	2	107	90	123	1.16	1.58
CEMB 666	1	78	72	74	1.03	1.03
Eagle1	1	50	46	59	1.28	1.28
MNH 988	3	151	118	167	0.83	1.84
NIAB 999	1	50	39	39	1.0	1.0
SG1	1	47	33	42	1.27	1.27
FH 142	3	151	133	153	1.13	1.21
IUB 2013	1	50	25	40	1.6	1.6
Lalazar	3	158	129	177	1.27	1.42
MNH 886	2	102	76	100	1.0	2.5
MNH 992	1	57	23	32	1.39	1.39
Grand total	**30**	**1549**	**1230**	**1698**	**20.6**	**27.8**
Average	**1.8**	**91.1**	**72.4**	**99.9**	**1.2**	**1.6**

The research and organizational weekly reports on the occurrence of pink bollworm above/below EIL were received during the season (May to October) this year (2014). Based on these reports, detailed study was conducted and was found in consistent with these reports. It is evident from the results of this study that infestation of PBW was significantly higher from all the three tesils/subdivisions of the district but Kehror Pacca was the most affected tehsil. It might be because of the reason that there was maximum area under early cotton (sown in March) which served a source for survival and multiplication of PBW. Secondly, it is near from the Indian border where resistance of PBW has already been reported (Sharma, 2010; Dahi, 2012, Shahid, 2014; Monsanto, 2015) and the resistant population may be spreading gradually. A number of missteps and risks were already highlighted by the reporters (Spielman *et al.*, 2014) including loop holes in the regulation and quality standard assurance which could lead to early resistance of bollworms. This study confirms their apprehensions.

Researchers (Ali, 2013; Spielman *et al.*, 2014) had also reported scarcity of the refuge alternate plants and non Bt cotton crops in cotton growing areas of Pakistan and concluded this factor as important one in the development of resistance in bollworms. Improper resistance management was envisaged in these reports which support the results of our study. The infestation recorded on all seventeen cultivars and from all three subdivisions included in the study, also points out the presence of a resistant population of PBW. A number of researchers (Dennehy *et al*., 2004; Tabashnik and Carrière, 2004) had reported that PBW has been found susceptible to Bt toxin due to its development in refuge area. In the study areas as well as in other cotton zones in Pakistan, little or no measures of refuge-management were undertaken that may be resulted in the development of possible resistance in PBW

against Bt toxin through process of natural selection. The present unexpected infestation of PBW recorded on Bt cultivars in present study might be the result of resistance developed because of a tremendous selection pressure on natural population of the pest. The Bt-cotton cultivars/lines selected in present study had Cry1Ac gene makeup which demonstrated least resistance against PBW population. These results are in agreement with researchers and media reports documenting resistance in PBW to Cry1Ac (Tabashnik *et al*., 2008; Tabashnik *et al*., 2009; Stolte, 2014, Shahid, 2014, Monsanto, 2015). There are some reports (Hackle, 2011) about modification of Bt genes which have effective lethal effect on bollworms but these varieties are not yet commercially available in this country hence it cannot be compared in this study.

CONCLUSION

In case of Bt cotton, farmers in developing countries have not adopted the recommended precautionary measures like keeping refuge crop, maintenance of LC_{99} level of Bt proteins. Ignorance of our cotton stakeholders from these recommendations has enabled pink bollworm to develop and exhibit resistance against 17 commercial Bt cotton cultivars/varieties/lines sown in farmer fields in district Lodhran and tested in this study. It needs an immediate attention of the researchers for remedial measures.

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