Incipient infestations and threat of pink bollworm *Pectinophora gossypiella* (Saunders) on Bollgard-II cotton in the northern cotton-growing zone of India

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Pink Bollworm (PBW) incidence on Bollgard-II (BG II) cotton was not observed so far in North Zone of India. During 2018–19 cotton season, BG II cotton was found infested with Pink Bollworm at one location in Haryana and two locations in Punjab near cotton ginning and oil extraction units. Cotton seeds are being procured from the central and south India by many units where PBW has already acquired resistance against BG II hybrids. Bio-ecological and resistance monitoring studies of larvae collected from the infested location revealed absence of larval diapauses in surviving population and resistance levels equivalent to central/south zone populations. The threat perception of pink bollworm in north zone on BG II cotton due to transport of resistant larvae along with cotton seed is discussed.

Keywords: Cotton seeds, diapause, infestations, pheromone traps, pink bollworm.

AFTER the introduction of transgenic cotton in 2005 (ref. 1) in northern zone of India and subsequent approval of dual genes hybrids in 2006, cotton crop was protected from bollworms, especially the American bollworm, *Helicoverpa armigera* (Hubner), spotted bollworm, *Earias* spp. and pink bollworm (PBW), *Pectinophora gossypiella* (Saunders). In the northern cotton-growing zone of India comprising Punjab, Haryana and Rajasthan, cotton crop (*kharif*) is of 165–180 days duration (May–October) followed by wheat (*rabi*) sowing. The confined crop duration along with management of cotton stalks has helped in the destruction of surviving population of insect in leftover cotton stalks after harvest to avoid its carryover to next season. However, in non-*Bt* cotton genotypes, low to medium incidence of PBW was reported each year. The green boll destructive sampling done in non-*Bt* genotypes at various stages of crop growth from different locations of the northern zone recorded larval recovery ranging from 4.0% to 20.7% in 2012–13, 11–20.1% in 2013–14, 12.5–21.8% in 2014–15, 13.0–33.4% in 2015–16, 1.7–18.0% in 2016–17, 3.1–15.7% in 2017–18 and 3.33–12.43% in 2018–19 at 140, 160 and 175 days after sowing (Central Institute for Cotton Research, Sirsa, unpublished data). However, there was no report available for PBW survival on *Bt*-containing cotton genotypes from the northern cotton-growing zone of India from 2012 to 2018.

PBW is a monophagous pest of cotton and has developed resistance to Cry proteins in the central and southern zones of India[2]. Prior to evolution of resistance to *Bt* cotton, several field reports had documented drastic difference in field infestation and locule damage levels between *Bt* and non-*Bt* cotton hybrids in India[3]. Many factors could have aided in the evolution of resistance against *cry Bt*-toxin in PBW populations, especially the monophagous nature and reclusive feeding habit of the larvae. Field resistance to Cry1Ac *Bt*-cotton was noticed for the first time in PBW populations of Gujarat in 2009 (refs 4–6) and to Cry1Ac + Cry2Ab (Bollgard-II; BG-II) in 2014 (ref. 7). PBW larval incidence on BG-II cotton was not reported in northern India, but in central and southern India, the annual average PBW larval recovery from BG-II cotton was as high as 28.85–72.49% during 2014–2017 (ref. 2). In central and southern India, LC_{50} of Cry1Ac increased from a mean of 0.330 μg ml^{-1} in 2013 to a mean of 6.938 μg ml^{-1} in 2017. The LC_{50} value for Cry2Ab increased from a mean of 0.014 μg ml^{-1} in 2013 to a mean of 12.51 μg ml^{-1} in 2017. High PBW larval recovery on BG-II in conjunction with high LC_{50} values for Cry1Ac and Cry2Ab in major cotton-growing districts of central and southern India provides evidence of field-evolved resistance in PBW to *Bt*-protein in BG-II cotton[2,8]. In the northern zone, during the cotton-growing season of 2018–19, feedback was received from the farmers of Palwan village, Jind district, Haryana regarding bad opening of bolls in cotton fields. On investigation, it was found to have severe infestation of PBW. The bad boll opening or unopened bolls were observed in all the five BG-II hybrids cultivated by the farmers in an area of around 4.0 ha. The bad opening due to incidence of PBW was mainly confined around a cotton ginning-cum-oil extraction unit situated close to the infested field. Highest incidence of PBW, i.e. 100% larval recovery from infested green bolls and 35% opened bolls infestation was observed in the field located in front of the ginning mill, followed by an above economic threshold level incidence (10–12% opened bolls infestation) in two fields located adjacent to the ginning mill. However, PBW incidence showed declining trend in the fields as one moved away from the ginnery. The incidence persisted only up to 1 km radius in the low form/traces and there after became almost negligible. The trait purity of the hybrids was confirmed using *Bt*-strip test. The farmers informed us that the incidence of PBW was noticed in the same field during 2017–18 as well, but due to unawareness of the

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incidence of PBW, no efforts were made for its management which resulted in pest perpetuation.

In another instance, scientists at Punjab Agricultural University (PAU), Regional Research Station (RRS), Bathinda, Punjab also confirmed 27.76% open boll damage due to incidence of PBW on BG-II cotton hybrids in Jodhpur Romana village, Bathinda district, Punjab, during the first fortnight of November 2018–19. The infested plants were tested for Cry1Ac and Cry2Ab using later flow strip test. It was found that all plants tested positive for the presence of both the genes. It was also observed that this field was close to a cotton ginning-cum-oil extraction unit. Another nearby field also recorded incidence of 5.53% PBW open boll infestation, however, adjoining fields on the other side of the cotton ginning mill did not report any incidence of PBW. Similarly, experimental area of PAU, RRS, Bathinda, also recorded 12–27% open boll infestation of PBW on BG-II hybrids, in addition to non-*Bt*-cotton varieties. Incidentally, this field was also located near an oil extraction unit.

To ascertain the role of ginning and oil extraction units in PBW infestation, its incidence was monitored in cotton fields located adjoining some of ginneries-cum-oil extraction units of Haryana. No PBW infestation was recorded in fields situated around these ginneries**.** Similar surveys were conducted in BG-II cotton fields around some ginneries in northern Rajasthan and Punjab, and no PBW infestation was recorded.

The spread of PBW from its place of origin (India/Australia) to other countries of the world was mainly through the export of cotton in the form of seed cotton or cotton seed. During interaction with some of the ginning and oil extraction mills owners, it was brought to our notice that to keep the mills in operation round the year and to earn better profit, some of the ginners are procuring/purchasing cotton seeds from the central and southern Indian states, especially Gujarat, Andhra Pradesh and Telangana. The quality of the cotton cake as well as the percentage of oil extraction from the procured seeds were comparatively superior to the locally available cotton seeds. In case of the cotton ginning mill-cum-oil extraction unit at Jind the cotton seeds were procured from Telangana during May–June and stored in the open till these were used for oil extraction. There is a possibility that adults of PBW emerged and escaped from these cotton seed heaps into the open environment and infested the cotton crops grown in vicinity. During a visit to the ginning and oil extraction unit, it was also noticed that the leftovers of cotton seeds, after ginning and oil extraction, heaped in front of the unit near to field reported highest infestation which confirms the possibility of emergence and spread of adults of PBW through infested cotton seeds in the ginning-cum-oil extraction unit in the reported field.

A team of scientist from ICAR-Central Institute for Cotton Research, Nagpur and its Regional Station, Sirsa collected surviving larvae from the BG-II infested field to conduct bio-ecological and resistance monitoring studies. Bio-ecological studies on surviving larvae recovered from BG-II cotton in the northern zone (Palwan village), were conducted during October–December 2018. Larvae recovered from non-*Bt* genotypes from Sriganganagar, Rajasthan, in the same zone were also studied for comparison. The results of the study revealed that PBW larval duration (31.93 ± 13.78 days) was short in case of larvae collected from BG-II surviving population, whereas it was very long (72.57 ± 34.72 days) in the population collected from non-*Bt* fields, i.e. Sriganganagar due to the habit of larval diapause of the northern Indian population. This indicated that the surviving population collected from Jind was different from the northern zone population as it must have been brought from locations where PBW's life cycle is short as pest does not undergo diapause[8], i.e. the southern zone. For further confirmation, resistance monitoring studies were conducted on the larval population collected from the non-*Bt* and BG-II surviving populations, which also indicated that LC_{50} of Cry2AB of the northern zone population ranged between 0.021 and 7.493 μg ml^{-1}. The lowest LC_{50} of Cry2Ab (0.021 μg ml^{-1}) was obtained from Abohar (non-*Bt*) population of the northern zone, whereas the highest (7.493 μg ml^{-1}) was from the Jind BG-II recovered population. Monitoring through pheromone traps has begun in and around the ginneries, especially in Jind, where PBW infestations were first detected. The observations were recorded till December 2018 in the ginning and oil extraction mill and fields adjoining to it. Very less emergence of moth was recorded in the mill till 12–18 November 2018, but good trap catches were recorded during the monitoring period until December 2018 in the adjoining fields though no moth trap catches were recorded thereafter. Maximum trap catches were recorded in the field located in front of the mill having highest PBW infestations. However, the pheromone traps installed at some other ginneries in Sirsa district, Haryana, recorded no trap catches.

The critical analysis of development of resistance in PBW to *Bt*-toxin in the central and southern Indian cotton-growing states vis-à-vis no PBW incidence in the northern Indian cotton-growing states during the past 12 years signifies the following: (i) In North India, the sowing season is short, i.e. from 15 April to May whereas in Central and South India, the sowing season is very long, i.e. from April (under irrigated condition) to July (under rainfed condition), thus providing different phenological phases of crop growth for survival and multiplication of PBW. In Punjab, Pakistan, staggered sowing of cotton has facilitated the survival and multiplication of this insect-pest[9]. (ii) In North India, mostly cotton–wheat rotation is followed; therefore, cotton crop is terminated by the end of November, whereas crop duration is extended beyond December (until February–March) in Central and

South India, resulting in the survival of PBW in fruiting parts for a longer period. Such extended phase intensifies *Bt*-toxin selection pressure and resistance development gets accelerated. (iii) Almost all the cotton sticks are removed from the fields and utilized as firewood in households or industries in North India. However, in South India majority of farmers either collect or bury cotton stalks in the fields, except a few who incorporate them in the soil after shredding. In Central India also the same practice is followed, but some farmers maintain ratoon if the irrigation facilities are available and also allow animal grazing on the cotton stubbles after harvesting.

Reports of PBW infestation during the cotton-growing season in North India must be taken seriously and efforts for its management need to be intensified to prevent further spread in non-PBW-infested cotton areas. The interstate movement of cotton seeds, as apparently evident, must have helped in the transportation of resistant PBW larvae surviving in cotton seeds and that has spread to the fields adjoining the ginneries, where cotton seeds have been stacked and stocked for a longer duration, especially in the open. PBW in the northern zone warrants restriction to be imposed on inter-zonal movement of cotton seeds and the possibilities of movement of hidden infestation of suspected pests and diseases. Similar to cotton seed, seed cotton trade mainly within the state or within the zone, especially in the bordering towns must be done with utmost care. Though movement of seed cotton being bulky in nature is not preferred among states of different zones, but, if practised, this can also pose a threat of introduction of resistant larval populations/infestations in new areas.

There is an urgent need to monitor the emergence of moths in the ginneries, especially through pheromone traps to ascertain the possibilities of carryover of PBW larvae and subsequent moth emergence, which may pose a threat of introduction of resistant larvae in the northern zone. In addition, fields adjoining the ginning-cum-oil extraction units should be effectively monitored through pheromone traps, green boll destructive sampling and assessment of open bolls and locule damage to restrict further spread of PBW in the northern cotton-growing states of India.

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Identification of Indian butterflies and moths with deep convolutional neural networks

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This paper reports our efforts to use artificial intelligence based on deep convolutional neural network (CNN) as a tool to identify Indian butterflies and moths. We compiled a dataset of over 170,000 images for 800 Indian butterfly species and 500 Indian moth species from diverse sources. We adopted the EfficientNet-B6 architecture for our CNN model, with about 44 million learnable parameters. We trained an ensemble of 5 such models on different subsets of the images in our data, employing artificial image augmentation techniques and transfer learning. This ensemble achieved a balanced top-1 accuracy of 86.5%, top-3 accuracy of 94.7%, and top-5 accuracy of 96.4% on the 1300 species, and a mean F_1 score of 0.867. Thus, our efforts demonstrate artificial intelligence can be effectively used for identifying these biological species that would substantially enhance the work efficiency of field level biologists in several spheres of investigations.

Keywords: Artificial intelligence, butterfly identification, convolutional neural network, moth identification.

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