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Indian scenario on the occurrence of a dreaded insect pest Pink bollworm, Pectinophora gossypiella on Bt cotton-A review

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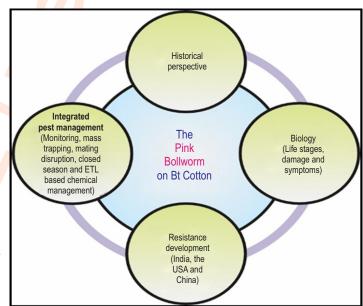
Abstract

The pink bollworm, Pectinophora gossypiella, has become a significant production constraint on Bt cotton in India. This problem is unique to India because the pest has developed multi-fold resistance to Cry toxins in many Indian populations but not in other countries.

Most Indian populations have developed multifold resistance to Cry 1 Ac and Cry 1Ac + Cry 2 Ab toxins. Year-round cultivation of long-duration Bt cotton hybrids on a large scale has a pronounced impact on the incidence. Also discussed other factors responsible for the occurrence of pink bollworm on Bt cotton in India. Insecticide Resistance Management (IRM) strategies implemented by different cotton-growing countries globally; the USA, India, and China had a significant impact on the interaction of pink bollworm on Bt cotton.

Huge selection pressure resulted in resistance to Cry toxins. Time-tested IPM, if implemented on a community basis focusing on pheromones technology and closed season, will help sustain the cotton cultivation in India in the future. Thus, this review aims to congregate exhaustive information on the history, biology, resistance to Bt cotton, and Integrated Management (IPM) options for the Indian scenario, which would help researchers in their future endeavors.

Key words: Bt cotton, Cry toxins, Management options, Pectinophora gossypiella, Pink bollworm



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Introduction

The pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae) is one of the dreaded insect pests on cotton and ranks among the top six most important insect pests of the world (August Busck, 1917). Maxwell-Lefroy and Howlett (1911) indicated that the minimum loss in India was more than a million dollars due to this pest. During 1915 in the Hawaiian Islands, cotton cultivation was abandoned due to this pest. Between 1965-1985 the cotton growers of Imperial valley of California lost as much as one-third of the crop to PBW damage, suffered a decreased lint quality, and paid a significant amount of crop income back as insecticide cost (up to \$300/acre) (Thomas Miller, 2001). It is the most harmful cotton pest because it is difficult to control with insecticides. Many eggs are laid on the sutures or under the bracteoles near the boll's base, particularly bolls up to 14 days old. Soon after hatching, larvae can penetrate flowers or bolls within 20-30 minutes (Hutchinson et al., 1988) or two hours (Ingram, 1994).

Sometimes due to application of several sprays has resulted in the outbreaks of secondary pests, otherwise regulated by natural enemies. The species was described first in India (Saunders 1843 and 1851). It was destructive to the American cotton grown in India but seldom attacking the native cotton. After reporting from India in 1843, it spread to different countries through cotton trade. It arrived in the USA (Texas) in 1917 from Mexico. Later the species spread to all the cotton tracts. Now, it is a cosmopolitan pest reported from most of the world's cottongrowing countries. Everything about the biology of the PBW is geared to survive. For example, the species can undergo diapause, but a specific population does not. Further, the trigger for diapause in PBW is determined by biotic factors and the host's condition, reinforcing the concept that PBWs are survivors (Thomas Miller, 2001). Pink bollworm, popularly called a pinkie in America, is a micro-lepidopteran insect 0.5 cm long and weighing 20 mg. Mostly it invades cotton (Gossypium) but has also been found on plants related to the Malvaceae, vegetable okra, and ornamental hibiscus. It is a tropical insect, and some populations can undergo diapause during the period when food resources are scarce.

Transgenic cotton, which produces *B. thuringiensis* (*Bt*) toxins, was effective against *Helicoverpa armigera* (Hubner), *Pectinophora gossypiella* (Saunders), *Earias vitella* (Fabricius), and *Earias insulana* (Boisduval) (Khadi *et al.,* 2001; Kranthi, 2001; Venugopal and Ramaswami, 2002 and Kranthi *et al.,* 2004). In India, *P. gossypiella* incidence has been on the rise. Patil *et al.* (2007) reported an increase in locule damage in the Raichur area of Karnataka from 44.8% during 2001-2002 to 62.6% in 2004-2005. A high incidence of *P. gossypiella* was reported in Haryana (Kumar and Saini, 2005). The All-India Coordinated Cotton Improvement Project (AICCIP) reported high catches of *P. gossypiella* with pheromone traps in Gujarat, especially at Surat and Junagarh, during the last week of November 2009 to January 2010, and in Rajasthan at Sriganganagar from August to October

2009, as compared with ten other locations in the country. Larvae feed inside the boll on developing seeds, thus reducing yield and lint quality with pink-yellow spots of its body coloration. Being an internal feeder, it is less amenable to insecticidal control. Bt-cotton expressing Cry1Ac is highly effective against bollworms, including *P. gossypiella*. Benedict *et al.* (1996) reported a higher efficacy of Bt cotton in providing management of pink bollworm compared with other lepidopterans such as tobacco budworm, *Heliothis virescens*, cabbage looper, *Trichoplusiani*, and beet armyworm, *Spodoptera exigua*. Commercially available varieties of Bt cotton are highly effective in controlling pink bollworm in the United States (Simmons *et al.*, 1998; Henneberry *et al.*, 2000; Tabashnik *et al.*, 2002; Carrier *et al.*, 2003) and in India (Jeughale *et al.*, 2007; Nadaf and Goud, 2007).

However, on 6th March 2010, Monsanto India Limited reported pink bollworm resistance to Bt cotton producing Cry1Ac planted in 2009 under field conditions in four districts of Gujarat, namely Amreli, Bhavnagar, Junagarh, and Rajkot, based on bioassays and the presence of larval incidence and damage to Bt cotton (Mohan et al., 2015). The propensity of pink bollworm to evolve resistance to the selection pressure of Cry1Ac has already been reported (Bartlett 1995; Tabashnik et al., 2000, 2004 and 2006). Tabashnik et al. (2009), even reported asymmetrical cross-resistance. During the 2015 season, a team of scientists from the ICAR-CICR, Nagpur, visited cotton fields in Gujarat and reported PBW incidence on Bollgard II cotton, mostly in green bolls (second picking) in districts; Junagadh, Bhavnagar, Amreli, and Bharuch. In Andhra Pradesh, PBW Incidence on Bollgard II cotton was reported from all the cotton-growing tractsfrom 2015-16 season onwards (AICCIP 2015-16 and 2016-17).

The survey conducted in 16 major cotton growing districts of Maharastra revealed the widespread incidence of Pink bollworm on Bt cotton to the extent of 40-90%, which accounted for anticipated yield losses between 20-30% (Fand et al., 2019). It has caused severe damage to Bollgard II cotton in Andhra Pradesh in 2020. Yield loss to 50% was recorded in certain pockets (Prasada Rao and Kranthi, 2021). The pest had developed multi-fold resistance to Cry 1Ac and Cry 2Ab toxins in India (Naik et al., 2018). Surprisingly, the problem is unique to India and Pakistan. Major cotton-growing countries like China, the USA, and Australia are ableto control the PBW with Cry toxins. Thus, this review aims to accumulate comprehensive information on the history, biology, resistance to Cry toxins, and Integrated Pest Management (IPM) strategies for the Indian scenario, which would help the researchers involved in PBW management.

Life stages of pink bollworm, Pectinophora gossypiella: The eggs are laid singly (Fig. 1a) or, more commonly, in small groups (Fig. 1b). The eggs are tiny and difficult to detect without a magnifying lens. The egg measures about 0.5 mm in length and 0.25 mm in width. Early in the season, eggs are placed in sheltered places, plant axes of petioles or peduncles, underneath young leaves, buds, or flowers (Vennila et al., 2007). But most

commonly found near the apex of green boll in slight longitudinal depressions, which indicate its divisions. Single to four eggs (Fig.1c) are widely observed, and sometimes as many as 20 may be found on a single boll, probably laid by several females. The number of eggs laid by a single female is difficult to ascertain in nature, but dissections prove that each female can lay more than 100 eggs. The egg hatches in 4 to 14 days after it is laid (August Busk, 1917). The larva, when first hatched, is very small, glassy white, with a light brown head, thorasic shield, and tubercles. White caterpillars with dark brown heads are due to sclerotized prothorasic shield (Vennila et al., 2007) (Fig. 2a).

The larva is easily overlooked at this stage when the boll is opened and examined. The mature larva is 10-12 mm long and has broad horizontal bands of pink color (Fig. 2b). Grown-up larvae assume strong pink suffusion, which has popular name "Pink Bollworm" (August Busk, 1917). The larval cycle lasts for 9-14 days in hotter regions. The mature larvae have a shortcycle to pupate or a long-cycle to enter a diapause state. Short-cycle is a common phenomenon in South India, and diapause is observed in north and central India. Short cycle larvae pupating may cut a round exit hole through the carpel wall and fall onto the ground or tunnel the cuticle leaving it as a transparent window and pupate inside. The long-cycle larvae entering diapause spins a tough thick-walled, closely woven spherical cell called 'hibernaculum with no exit hole.

Always the long-cycle larvae occur during the end of crop season, where there are mature bolls present, and larvae often form their hibernacula inside seeds. Hibernacula may occupy a single seed or double seed. Diapause larvae often spin up in the lint of an open boll and, if still active in ginnery, spin up on bales of lint, bags of seed, or cracks and crevices. Moths emerging from the hibernating larvae are long-lived, with females and males alive for 56 and 20 days, respectively (Vennila et al., 2007). Pupae is light when fresh, gradually becomes dark brown as pupation proceeds (Fig.3a). Pupa measures up to 7 mm in length. The pupal period lasts for eight to 13 days. Pupation occurs inside a loose-fitting cocoon with a highly webbed exit at one end (Vennila et al., 2007). Adults are small, greyish brown with blackish bands on the forewings, and the hind wings are silvery grey, inconspicuous moths (Fig. 3b). When their wings are folded, they have an elongated appearance.

The wingtips are conspicuously fringed. The moths are about 7-10 mm with a wingspan of 15-20 mm. Moths emerge during morning or in the evening but are nocturnal, hiding amongst soil debris or cracks during the day. They emerge from pupae in approximately 1:1 male-to-female ratio. Two to three days after emergence, females mate and prepare to lay eggs. The flight time is 6.30 to 8.00 p.m.; though they have strong wings for flying, they fly only to the nearest cotton bolls for copulation and egg-laying, which under normal conditions takes place soon after issue. The moths die shortly after oviposition. Adults live 14-20 days, sometimes extends up to one month. Generally, the life cycle completes in 40-50 days. During cooler season, life extends

to 3-4 months. Thus, four to six overlapping generations are produced in a year.

Nature and symptoms of damage: The larva is exclusively an inside feeder within the boll and does not attack the cotton leaves or shoots. Sometimes young larva attacks the bud and found in the flowers' ovary, devouring the tender ovules and preventing lint formation. Such larva rarely attains their total growth in the flower but migrate to a boll for their later support (August Busck 1917). When larva attacks 10-days-old buds, shedding of bud is observed, and larva dies. But larva completes development when it strikes older buds. Larva in flower bud spins webbing prevents proper flower opening leading to "Rosette Flowers" (Vennila et al., 2007). Ten to twenty-days-old bolls are attacked from under bracteoles after hatching larvae tunnels into the boll under the eggshell or nearby and feed at the beginning of soft inner walls in equally soft partitions separating the divisions of boll. The larvae are easily overlooked at this stage when the boll is opened and examined. Still, the minute entrance hole and eggshell indicate infestation. The boll's dissection reveal small larva mining the wall, at this stage it is a challenge to discriminate between infected and uninfected boll, except for discovering eggshell and entrance hole on the infested boll.

Later, considerable individual variation in the further course of attack was seen, partly depending on the location of the egg and condition of the boll and somewhat depending on the direction of individual larva (August Busck, 1917). Generally, larva bores near the boll's apex and tunnels through the wall to the base invading the lowest seeds and proceeds to the next seed, eventually ending as a full-grown larva in one of the seeds near the boll's tip. Sometimes opposite movements may take place. A larva may confine to a single section of the boll but invade the adjoining section, or sometimes all sections are equally attacked by a single larva. In any case, invaded boll becomes unfit. The larva the moves into another boll on which a large, conspicuous, and frass surrounded hole can be observed. The larva eats the seeds, and tunnels and soils the lint, arresting the growth, rotting, premature and imperfect opening of the boll is observed. Uninfected parts of the boll are also retarded in development due to the attack. When two or more larvae attack the boll, total loss of seeds and lint is often not uncommon. The larva typically makes its cocoon, and pupates within the boll may be within the last seed attacked.

Before finishing the cocoon, the larva gnaws a round hole through the boll's outer wall to ensure a free exit for the issuing moth. If disturbed at the time of maturity, the larvae may leave the boll, fall to the ground, spin its cocoon an inch or more down in the soil or any other convenient shelter, and complete the transformation. Under normal conditions, the pupation always takes place within the boll. The empty pupal case remains in the cocoon when the moth emerges. The infected flowers do not open fully, and they get twisted (Rosette flowers, Improper opening of petals). The presence of black spots on the green boll may often indicate PBW damage. PBW-damaged

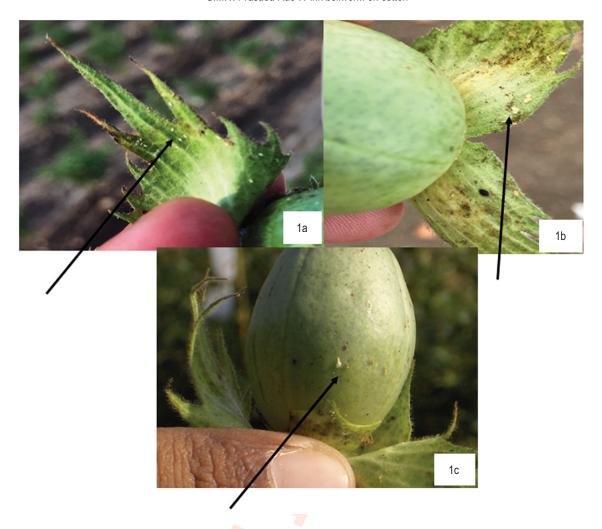


Fig. 1: (a) Single egg, (b) Eggs in small groups and (c) Single to 4 eggs on a boll.

bolls often predispose to bacterial infection, resulting in blackening of the boll rind from outside. Stained lint around feeding areas resulting in inferior quality seed cotton is observed in open bolls. A small hole of 1.5 to 2 mm diameter indicates the exit of insect from the boll (Exit holes on green bolls).

Development of resistance to *Bt* **cotton:** Naik *et al.* (2018) reported nil larval incidence of PBW on *Bt* cotton in North India. In Central and South India, larval recovery in Bt cotton ranged from 29 to 72% during 2014-2017. Likewise, the mean resistance ratio for Cry 1Ac was 47 (18-127) during 2013 and increased to 1387 (704-2060) during 2017. A similar increasing trend was observed for Cry 2Ab also. Mean resistance ratio increased from 5.4 (1-31) in 2013 to 4196 (1306-9366) in 2017. High level of resistance to Cry 1Ac (371.8 fold) and Cry2 Ab (4214.3 fold) recorded in the late season Rajkot populations (Naik *et al.*, 2021). Tabahsnik and Carriere (2019) reviewed the global resistance monitoring data. It indicated that resistance management strategies adopted by three major cotton-growing countries globally, the USA, China,

and India, had a significant impact on *Pectinophora gossypiella* with Bt cotton. They primarily pointed that the abundance of refuge varied among these three countries that might have played a key role in striking differences in the same pest species incidence on the same crop and same toxins. Further, PBW populations in all the three countries contain cadherin mutations responsible for Cry 1 Ac resistance (Morin *et al.*, 2003; Fabrick *et al.*, 2014; Wang *et al.*, 2019) and ABC transporter mutations in the population of USA and India conferring resistance to Cry 2 Ab (Mathew *et al.*, 2018). The partial mitochondrial COI sequence analyses of 214 pink bollworm populations from 9 cotton-growing states of India indicate population expansion in India.

Resistance to Cry toxins does not impact mt DNA in population of pink bollworm. Two populations of pink bollworm, those occurring early in the season, are genetically close to late-season populations concerning their CO1 region (Naik *et al.*, 2020). Certain influencing conditions like maximum temperature higher than 33°C, morning relative humidity less than 70%, evening



Fig. 2: (a) White larva with sclerotized prothorasic shield and (b) Pink color larva with bands.

relative humidity higher than 40% during standard weeks of 40, 41 and 43 and minimum temperature less than 12°C between 48 and 49 standard weeks enhances the severity of Pink bollworm on cotton (Vennila and Biradar, 2007).

Occurrence of pink bollworm on Bt cotton in India: This problem is confined to India and Pakistan. No other country in the world faces resistance development in pink bollworm to Cry 1 Ac and Cry2 Ab. It is interesting to note that none of the other 14 Bt cotton cultivating countries are facing the problem. China still successfully controls pink bollworm with first-generation Bt cotton. The USA and Australia have moved to 3rd generation Bt cotton without having any resistance issues to pink bollworm. Why this unique disaster to India? What are the possible reasons? Practically zero compliance with the refuge strategy, a critical Insecticide Resistance Management (IRM) approach considered as crucial in delaying development resistance in target pests, a large proportion of Bt cotton area (> 95%), and year-round cultivation of long duration Bt hybrids are the major driving factors for high selection pressure and eventual development of resistance in PBW (Prasada Rao and Kranthi 2021). A field survey conducted in 2018 also reported very high flower damage (29.5%) by PBW in Andhra Pradesh (Raju et al., 2021). Furthermore, PBW is an indigenous insect pest; a quick amalgamation of alleles with several adaptive mechanisms for resistance to Bt toxins could have fast-tracked Bt resistance populations in PBW over other polyphagous bollworms (Ojha et al., 2014). The mode of resistance in PBW arises from mutations in the cadherin gene leading to low or less binding affinity.

The Indian PBW populations exhibited eight novel mutations in the cadherin gene, severely disrupting cadherin alleles responsible for Cry 1 Ac resistance. These are different from four mutations found in laboratory-reared PBW-resistant

populations in Arizona. DNA (cDNA) analysis of these severely disrupted alleles revealed a total of 19 transcript isoforms. Seven of these eight alleles produce two or more different transcript isoforms, indicating alternate splicing of mRNA. The first report of involvement of alternate splicing in field evolved resistance that reduced the efficacy of Bt cotton against PBW (Fabrick et al., 2014). Rishi Kumar et. al. (2021) conducted experiments on 'builtin-refuge' (BIR) where non-Bt cotton seeds were blended with Bt cotton seeds in various proportions of five, ten, 50%, along with 20% recommended structured refuge. Each treatment was evaluated in terms of fruiting body damage by major cotton lepidopterans, relative to the regulator stipulated 20% non-Bt structured refuge at five locations across India in Kharif 2012. They reported that treatments with five and ten percent non-Bt blends with bollgard-II were as effective as 20% structured refuge, based on fruiting body damage due to bollworms, a key factor in managing Bt resistance without any compromise to the seed cotton vield. It was reported that the seed blend technology is an excellent option to impose refugia compliance to delay resistance in Bt cotton to bollworms in a country having lesser alternate hosts available for the target bollworms during the season, and voluntary compliance of refuge is absent.

Non-occurrence of pink bollworm on *Bt* cotton in the United States of America and China: Pink bollworm is not a problem in the USA mainly because of the availability of different *Bt* cotton varieties for technology durability and improved insect resistance; Bollgard® 1 containing Cry 1Ac, Bollgard® II with Cry 1Ac + Cry 2 Ab, Widestrike TM with Cry 1 Ac + Cry 1F, Widestrike ® 3 contains Cry 1 Ac, Cry 1F, and VIP 3a, Bollgard ® 3 having VIP 3a along with Cry 1 Ac and Cry 2 Ab, Twinlight ® Cry 1 Ab and Cry 2 Ae, Twinlink ® plus containing Cry 1 Ab, Cry 2 Ae along with VIP 3 Aa 19, strict compliance of refuge, mass release of sterile PBW moths helped in sustaining *Bt* technology the USA and successful



Fig. 3: (a) PBW pupae and (b) PBW adults.

implementation of IRM/IPM strategies, including pheromone-based monitoring and management also played a role. In China, farmers are still growing *Bt* cotton containing Vry 1Ac, and they have not shifted to Bollgard II cotton. Farmers cultivated F2 hybrid seeds from crosses between Bt and non-Bt cotton, producing 25% non-*Bt* plants, which acted as a refuge in Bt cotton. Seed mixtures generated with F2 hybrids in China were effective against Pink bollworm. In 2011-2015, when F2 hybrid fields accounted for a mean of 67% of the total cotton cultivated and PBW population reduced by 96% and insecticide sprays reduce by 69% compared to 1995-1999 (non-*Bt* era) (Wan *et al.*, 2017). Further, short-season cotton cultivation might also delay the development of resistance to the pest (Dai and Dong, 2014).

Management strategies: Pink bollworm causes enormous loss by feeding on anther, style, developing bolls and seeds, resulting in unopened twisted flowers (Rosette flowers) and vigorous shedding of reproductive bodies. Incidence results in discoloration of lint, low oil content in seeds, and poor germination. In India, ratooning of cotton (Sharma and Mohindra 1948), late uprooting of cotton stubbles (Kulkarni et al., 1958), and staking of cotton stubbles for an extended period (Simwat and Sidhu, 1982) were identified as three primary reasons for survival and carryover of the pest and accounting for more than 85% of the pest population on new crop.

Closed season: The legally enforced closed season is to stop pest carryover. Cotton plants must be destroyed to create a Dead period or Closed period to prevent pests buildup. In Zimbabwe, the closed season is governed by the Plant Pest and disease act, which stipulates that any farmer who fails to comply will face a fine or imprisonment or both (Mubvekeri and Nobanda, 2012). Strict adherence to the Closed Season is the only available option for managing Pink Bollworm on cotton (CGA, 1998). Further, the

cotton handbook of Zimbabwe states that the minimum length for the closed season when no living cotton plants are allowed should be 65 days. The aerial parts of cotton plant should be destroyed by severing the stems below the first branch. Destruction of the plant must follow and complete before stipulated dates. In the USA, pink bollworm destroyed cotton in the Imperial Valley of California between 1965-1985. Eventually, farmers adopted a short-season strategy and successfully managed the menace pink bollworm on cotton in the valley. However, after introducing transgenic cotton that expresses endotoxin toxic to PBW, growers voted to abandon the short-season strategy (Thomas Miller, 2001). In India, in early 1911, cultural control in removing cotton sticks by the first August every year was made compulsory by law to minimize the incidence of pink bollworm on cotton in Madras State (CICR Technical Bulletin No. 8).

Application of pheromones: Pheromone technology is being used for monitoring and management through mass trapping and mating disruption techniques. Mating disruption is achieved when gossyplure is dispersed at high doses in the cotton canopy and can result in low damage levels. PB-rope dispensers provide a high doserate of pheromone release over a long period that significantly reduced moth catches in traps (Flint et al., 1985; Staten et al., 1987). A microencapsulated pheromone formulation has been applied aerially, but its effectiveness was reduced gradually, but comparable to insecticide sprays (Critheley et al., 1983; Lykouressis et al. (2005). Evaluated the mating disruption of pink bollworm by monitoring its population with pheromone baited traps as well as sampling flowers and bolls to record damage levels in cotton fields during 1988 and 1989 in Central Greece. The treated fields were compared with control fields in where 2-3 insecticide sprays were applied. During both years, the number of male moths caught in pheromone traps were significantly reduced in treated compared to control fields. Mating disruption reached 99.1%, 96.8% and 93.2% in different treated fields. However, the prevailing wind direction to cotton rows did not affect the percentage damage. Further, they reported a low incidence of sucking insect pests like whiteflies and aphids in the treated plots. Natural enemies' incidence might have regulated these insect pests generally found in relatively high numbers in cotton fields (Lykouressis and Perdikis, 1997 and Lykouressis et al., 2005). Similarly, Staten et al. (1987) indicated that mating disruption greatly reduces the possibility of late outbreaks of secondary pests. Finally, it was concluded that mating disruption played a key role in reducing pink bollworm catches in traps and lowering the damage. Jahnavi et al. (2019), assessed the Integrated Pest Management module and demonstrated mass trapping to manage PBW on cotton.

Other implementable integrated pest management **strategies:** The other implementable IPM strategies include. Deep ploughing exposes bollworm pupae to birds and excessive sun heat; Crop rotation to break the pest cycle; Promotion of short-duration single pick varieties (150 days) under high-density planting to avoid PBW; Practice balance application of NPK; Removal and destruction of rosette flowers dropped squares and pre-matured bolls to suppress the pest population; Avoiding synthetic pyrethroids spray up to 100 DAS; Release egg parasitoid, Trichogramma bractriae; After final picking, allow cattle, sheep, and goat to feed on immature green bolls and attacked bolls; The practice of gin and field sanitation, and Needbased spray of recommended chemical insecticides. Prasada and Kranthi (2021) emphasized that excess and indiscriminate use of organophosphate and neonicotinoid insecticides during early crop growth stages to manage sucking insect pests results in excessive vegetative growth and delays crop maturity since they are known to affect the changes in the physiology of cotton plant.

Considering the bitter experiences with Bollgard and Bollgard II cotton against PBW in India's Central and Southern cotton-growing states, there is an urgent need to proactively deploy new Bt cotton events containing other than Cry 1 Ac and Cry 2 Ab or modified toxins. Stringent implementation of Refugia as a part of Integrated Pest Management (IPM) strategy for newly deployed events. Further, aggressive adoption of pheromone technology in monitoring and managing PBW and strict compliance of "Dead season," at least for 120 to 150 days. That means no cotton cultivation between two seasons to break the pest's cycle will go a long way in sustainable cotton cultivation in India, more so in Andhra Pradesh.

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