# *Review Article*

# Resistance in Pink Bollworm *Pectinophora gossypiella* (Saunders) against Bt cotton, a major threat to cotton in India: A brief review

## Abstract

The pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) is a primary pest of cotton in many regions of the world. In many parts of India, pink bollworm (PBW) has become resistant to BG-I (Bollgard I®) that expresses a single Bt gene (Cry1Ac) in 2009 and BG-II (Cry1Ac and cry 2Ab) in 2015. The brief review addresses the possible reasons and solutions to resistance posed by this insect. The review addresses the following points: reasons for resistance in PBW is discussed; the life-history is discussed how it is different from other bollworms; the introduction of hybrid cotton lead to insecticide treadmill in India; reduced accomplishment of the refugia recommendations also contributed to the problem; poor compliance of the bio-safety laws also lead to the problem; unfavorable crop management practices prove to be an add-on to the problem. Furthermore, the possible solutions of the problem are discussed; monitoring and mating disruption; cultivation of High-density short season (HD-SS) pure line varieties; case studies of sterile insect technique (SIT) proved to be supporting in control of PBW; RNA interference technique can overcome the problem of resistance; use of chemical insecticides in different manner can change the perspective; proper cultural or farming practices can make the difference; Bio-control agents can overcome the problem; extension functionaries can help in solving the PBW problem. Therefore the management of resistance in PBW requires a broader vision and development of technology.

**Keywords**: Cotton, Bollworm, Hybrid, Resistance

## 1. Introduction:

Stepping back, the Genetic Engineering Appraisal Committee (GEAC) had approved the first transgenic single-gene *Bt*-cotton hybrids (Bollgard I®) in 2002 and the next-generation cotton transgenic with stacked *Bt* genes (Bollgard II®) called 'pyramids' in 2006 in India. The introduction of Bt cotton, between 2002 and 2006, reduced the use of insecticides from 40,672 tons[1] to the tune of 9,000 t[2]. Genetically engineered (GE) crops had proved to be potent tools of Integrated Pest Management (IPM) programs and important factor in improving the sustainability, economics, and social interactions among the growers[3,4], thus resulted into increase in area under GE crops since 1996, reaching 190 million hectares in 2016 globally[5].

Though Bt cotton proved to be a huge success, the sustainable use of this technique, need thorough understanding of the GE trait introduced, properties of the target crop, the cropping pattern and the socio economic standards are important for the successful integration of the GE crops into IPM systems[6]. One of the biggest challenges for sustainable use of technology is the evolution of resistance. The major reason behind the evolution of resistance is over-reliability on Bt crops without appropriate adoption of the Insect Resistance Management (IRM) or IPM practices[7,8]. One such example was resistance to Bollgard- I (Cry1Ac) in cotton against the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lep.: Gelechiidae), in India[9] and Bollgard II in 2015. Meissle[10] (2016) mentioned that GE crops should not be considered as the only solution to control pest. GE traits should complement a broader IPM strategy filled with a companion and compatible selective tactics, but should not remain the central focus for all the pest challenges in the system. It is equally crucial that other IPM practices are developed, optimized, and maintained for all crop pests[11].In this paper, we highlighted the possible reasons for the development of resistance in PBW and the challenges faced by the cotton industry. We also explained different IPM practices adopted in different cotton-growing countries all over the world based on the case studies and how they can be useful in Indian cotton production systems.

## 2. **Possible reasons for resistance in Pink Bollworm**

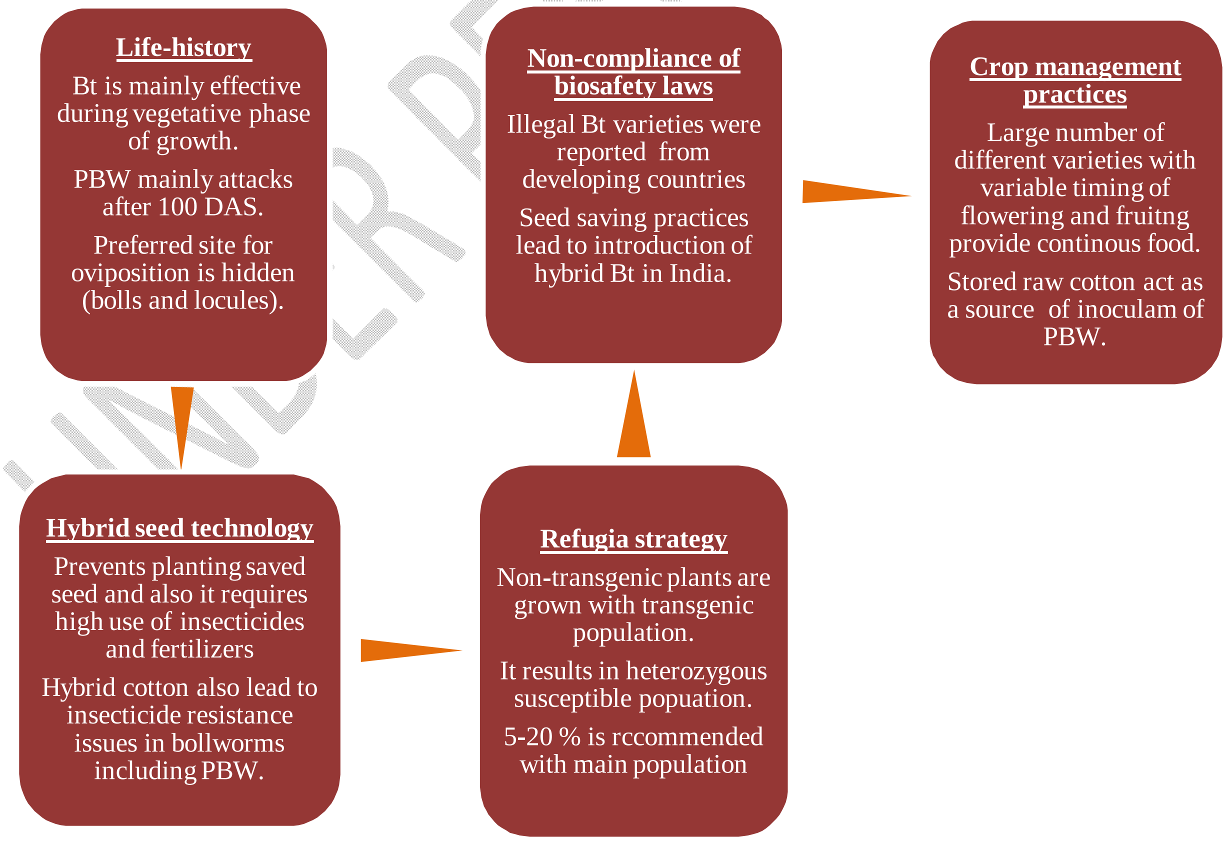
The resistance problem in cotton against Pink Bollworm is not attributed to a single factor, but it is an amalgamation of multiple factors. Following are the possible reason for resistance:-

### 2.1 Life –history

Life history theory is a very central and necessary part of both population ecology and general evolutionary theory, and it is especially useful in pest forecasting and management[12]. Identification of the pest, understanding its biology and seasonal population trends, damaging life stages and their habitats, nature of the damage and its economic significance, the vulnerability of each life stage for one or more control options, host preference, and alternate hosts, predictability of pest occurrence based on the environment, cropping trends, farming practices, and other influencing factors, and all the related information is critical for identifying an effective control strategy[13].

Early in the cotton season, PBW eggs are laid in the sheltered places of the plant axis of petioles or peduncles, the underside of young leaves, on buds or flowers but once the bolls are 15 days old, these are the most favorable sites for oviposition. The incubation period is 3-6 days. The larval cycle lasts for 9-14 days in hotter regions. The mature larvae are either 'short-cycle' or 'long cycle' differing according to the state of diapause. Short cycle larvae form a tunnel in the cuticle and fall to the ground by cutting a round exit hole through the carpel wall, leaving it as a transparent window and pupate inside the ground. Pupation takes place inside a loose cocoon with a highly webbed exit at one end. The pupal period ranges between 8 and 13 days. The life cycle is completed in 3-6 weeks. The late-season has invariably overlapping broods. On the other hand, the long cycle larvae enter diapause and spin up a spherical cells which is tough thick-walled, closely woven, referred to as "hibernaculum” with no exit hole. The long-term larvae always occur during the end of the crop season, where there are mature bolls present and larvae often form their hibernacula inside seeds. Hibernacula may occupy single seeds or double seeds. *P. gossypiella* hibernate as full-fed larvae during cold weather. Diapauses larvae often spin up in the lint of an open boll and if still active in ginnery, will spin up on bales of lint, bags of seed, or in cracks and crevices. Therefore, the long-lived larvae act as a source of inoculums and are more harmful. The PBW life-cycle differences than other bollworms could be the reason for early resistance in this pest:-

A. The effective population of PBW buildup starts after 100 to 110 days of crop emergence, while the peak infestations occur after 140 days, which coincides with the harvest of the crop[14]**.** The cry toxin expression levels in leaves decline after 110-120 days after sowing. Therefore, Bt-cotton controls bollworms effectively at 90-100% up to 100-110 days after sowing and 70-80% of the bollworm larvae thereafter. The reduction of Bt protein content in late-season cotton could be due to the over-expression of the Bt gene at earlier stages, which leads to gene regulation at post-transcription levels and consequently results in gene silencing at a later stage[9,15-17].



**Life-history**

Bt is mainly effective during vegetative phase of growth.

PBW mainly attacks after 100 DAS.

Preferred site for oviposition is hidden (bolls and locules).

**Non-compliance of biosafety laws**

Illegal Bt varieties were reported from developing countries

Seed saving practices lead to introduction of hybrid Bt in India.

**Crop management practices**

Large number of different varieties with variable timing of flowering and fruitng provide continous food.

Stored raw cotton act as a source of inoculam of PBW.

**Hybrid seed technology**

Prevents planting saved seed and also it requires high use of insecticides and fertilizers

Hybrid cotton also lead to insecticide resistance issues in bollworms including PBW.

**Refugia strategy**

Non-transgenic plants are grown with transgenic population.

It results in heterozygous susceptible popuation.

5-20 % is rccommended with main population

**Figure 1:- Reasons of resistance in pink bollworm.**

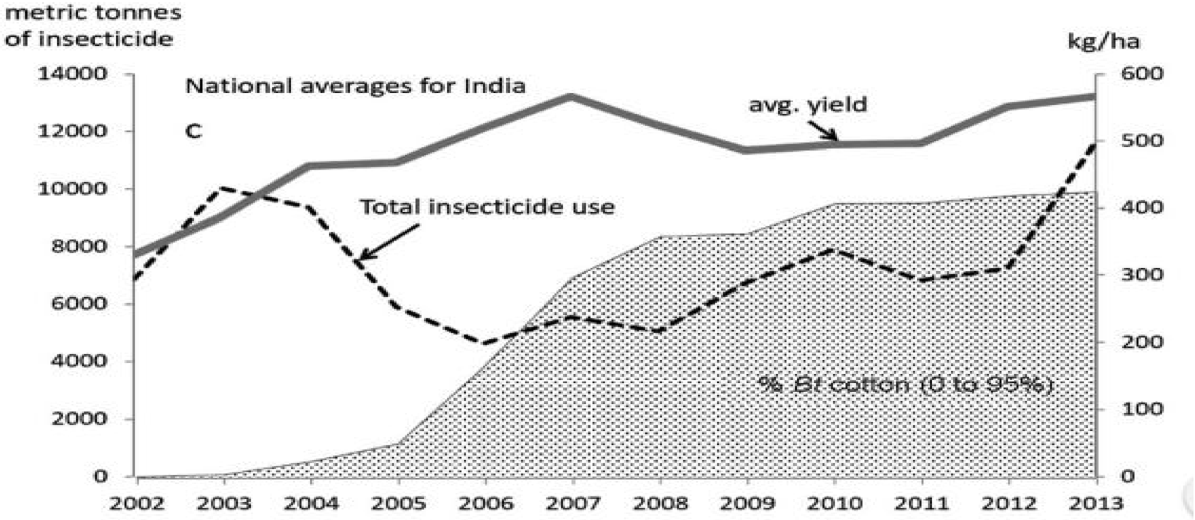


Figure 2: A summary of average national yield, insecticide use, and Bt cotton adoption in India (Gutierrez *et al*.,[19] 2017).

B. The preferred site for oviposition of PBW is bolls and locules where they are well protected and remain alive for many months, whereas *H. armigera* and *E. vitella* lay eggs on leaves. The bolls on F-1 plants contain seeds that segregate in a 3:1 ratio of Bt: non-Bt. Therefore bollworm larvae can survive on the 25% non-Bt seeds in green bolls. The pink bollworm survival in Bt-cotton is mainly due to the presence of such segregating Bt-cotton seeds in the green bolls of the Bt-cotton F1 hybrids[18].

C. As discussed earlier, late-season larvae enter in diapause, and incidence of *P. gossypiella* during the season commences from the moth emerging from the overwintering larvae through the summer season. This situation can be avoided by using high-density short duration varieties. Survival of the pest from one season to another is entirely through hibernating larvae in seeds, soils, and plant debris.

### 2.2 Hybrid cotton leads to pesticide treadmill in India

Cotton production in India has a 5000-year history but large changes started in 1790 when New World cotton (chiefly *Gossypium hirsutum* L. and later *Gossypium barbadense* L.) were introduced by the colonial British to feed their developing industrial revolution[20]. It's always been a debatable topic, why hybrid cotton was only introduced to India in the 1970s[21], but nowhere in the world, when it prevents planting a saved seed, besides it required a high use of insecticides and fertilizers[22,23]. The whole world grows highly fertile pure line varieties of *G.hirsutum*. The hybrid Bt cotton introduction provides initial relief to the farmers, however, the insecticides usage reached pre-2002 levels by 2013, as Bt cotton induced the outbreaks of new pests (e.g., plant bugs, whiteflies, mealybugs). Also, the introduction of hybrid cotton in India, ushered to the chain of problems, as excessive use of insecticides created wide ecological disruption and outbreaks of secondary pests that lead to yield losses and insecticide resistance issues in many defoliators including pink bollworms and other bollworms in the 1990s[9], hence complicated the insecticide-based cotton production system in India[24,25]. By 2013, the area under Bt cotton reached up to 95 percent. By this time, the pure line varieties disappeared from the market[21]and farmers got trapped in new hybrid technology treadmills[26].

### 2.3 Refugia strategy

Refugia strategy mainly focuses on the use of the biotechnological aspect of plant protection management, where an area consisting of non-transgenic plants is grown with a transgenic population, that supports sufficient homozygous susceptible insects to mate with the majority of homozygous resistant individuals, resulting in heterozygous susceptible progeny. Retrospective analyses of global resistance monitoring data lead to the assumption that refugees can substantially delay resistance to Bt crops[27-30]. The effectiveness of refuge strategy is governed by two key conditions: sufficient refuges of non-Bt host plants and a toxin concentration in Bt plants, that can kill all hybrid progeny, which was also called as ''high dose'' criterion[31]. Andow and Hutchison[32] (1998) stated that the high-dose refuge strategy demands that Bt plants express a sufficiently high concentration of Bt proteins so that 95% of the heterozygous individuals carrying one copy of a major resistance allele can be killed. 'Based on data, the US EPA Scientific Advisory Panel (SAP) on Bt Plant-Pesticides and Resistance Management[33,34] suggested that a working definition of the high dose should be 'a dose 25 times the toxin concentration needed to kill Bt-susceptible larvae.' According to the researchers, higher pink bollworm resistance is noticed in India and China as compared to the developed countries. Pink bollworm resistance to Bt cotton has been reported in the field in India, where farmer compliance with the refuge strategy has been low[9,35]. Wan *et al*[36] (2012) hypothesized that lower concentration of Cry1Ac in Bt cotton in these countries compared with the United States could be the reason for the acceleration of pink bollworm resistance in

India and China, that could lead to an increase in survival of heterozygotes and thus increase the dominance of resistance.

Earlier recommendations of non-transgenic crops specified that between 5 and 20% of any given area should be included as refugia for Bt-cotton[37-39]. Ministry of Environment, Forest and Climate Change, Government of India (GoI) had recommended sowing of 20% of the area with non-*Bt* cotton as a 'structured' refuge for both types of *Bt*-cotton[40], but compliance is very low. Indian Agriculture Research Institute (IARI) had recommended, 'refuge-in-bag' (RIB) with 95:5 (90-95% Bt seeds: 5-10% non-Bt seeds as permissible limits) and the non-Bt seeds must be of the near-isogenic hybrid corresponding to the BG-II hybrid[40].

To address the problem of insect resistance, Insect Resistance Management (IRM) programs have been proactively implemented wherever Bt crops have been commercialized, with these programs being mandatory in some countries including the USA, Canada, Australia, the EU, the Philippines, and South Africa[41]. The Australian cotton industry showcased one great story for the adoption of IRM. In the 1990s, Australian cotton growers were challenged with a high level of Lepidopteran resistance to insecticides, which almost led to the end of the cotton industry[42-44]. High awareness of the need for IRM by growers and appropriate education and training has resulted in refuge adoption that is consistent near to 100% in Australia.

Presently Bt cotton commercialization in India involves the supply of seeds of refugia separately along with seeds of Bt cotton. There are always changes in theory and practice that farmers will not sow refugia and grow only Bt cotton since the cultivation of refugia brings about a reduction of productivity to the extent of proportion of refugia in Bt cotton fields, resulting in lower compliance of mandatory refugia in India.

### 2.4 Poor compliance of Intellectual Property Rights (IPR) recommendations

Biosafety regulations can also have unintended consequences. Between 2002 and 2006, only one company in India – MAHYCO Monsanto Biotech (MMB) – got permission to sell the Bt gene implanted in cotton, and therefore, regulations in effect gave MMB a monopoly on the sale of legal Bt. However, farmers planted Bt cotton in India before the official approval, in 2002[45]. Despite the abundant resources and time invested in promulgating new laws and setting up new institutions for biosafety, illegal transgenic varieties were reported from many developing countries such as Brazil, China, and India[46-48]. A survey of 200 cotton farmers found that 60% of their cotton area in 2007 was under illegal Bt seeds[49]. Monsanto speculated that Cry1Ac concentration was lower in the unapproved Bt cotton than approved Bt cotton in India and that early use of unapproved Bt cotton ushered to the resistance problem.

The failure to enforce bio-safety laws is widespread and demands explanation. In India, farmers exhibit a tradition of seed saving, seed exchange, and seed experimentation that has historically produced better crops and better incomes. Authors like Herring[45] (2007) and Shah[50] (2005) have emphasized the limits of legal monopolies in seeds and suggest that farmers are empowered to make "gray market" versions of the legal seed. Many scientists have also suggested that non-compliance of seed laws by the farmers could be the reason why Bt is only introduced in hybrid seeds in India.

### 2.5 Crop management practices

Many crop management practices lead to an increase in the incidence of PBW. When cotton prices are hiked, farmers extend their crop up to April-May and this practice can provide continuous availability of cotton all through the year. The minor seasonal peak of PBW occurs in June-July and it coincides with the early (April-May) sown cotton crop at the time of flowering. As PBW is a winter pest, it mainly causes damage in November, which can be prevented. In the absence of the crop or the crop residues, the pupae enter in diapauses in December. However, if the crop is available beyond November, the pest continues to survive on the fruiting parts. This extended phase intensifies Bt-toxin selection pressure and resistance development is accelerated. Long term storage of raw cotton in ginning mills and market yards serve as a source of PBW inoculum to the ensuing crop[18].

## 3. Strategies for resistance management

### 3.1 Monitoring

The Mating Disruption (MD) technique works with the principle that the air in an agricultural field (e.g. orchard) is saturated with sex pheromone, which prevents male pests from locating females and thereby preventing the reproduction process[51]. This technique has been widely used successfully to control lepidopteran species. MD technique is widely used for the management of PBW[52]. In the case of pink bollworm, female release sex pheromone called gossyplure[53] and this pheromone has been used for monitoring and mating disruption studies to provide comparatively better control from insecticides[54-56]. Kranthi[18] (2015) recommended the use of ‘pheromone traps’ and ‘green boll dissection’ for regular monitoring and mating disruption at the rate of 8 moths per trap per night or 10% damage in green bolls. Boguslawski and Basedow[57] (2001) used the MD technique on cotton for PBW in a semi-arid region of Egypt and claimed that it was 52% more efficient than conventional methods. Lykouressis *et al*[58] (2005) reported that this technique was more effective in preventing damage by PBW if applied early in the cotton-growing season. Special care should be taken to monitor the emergence of moths in and around the ginneries with the help of pheromone traps to confirm the possibilities of presence of PBW larvae and subsequent moth emergence.

### 3.2 High-density short-season varieties

The solution to the insecticide treadmill and to prevent ecological hazard in Indian irrigated and rainfed cotton is short-season high-density non-hybrid non-GMO cotton with minimal insecticide use[21,26] the potential of which has been demonstrated by Indian scientists at the Central Institute for Cotton Research, Nagpur[59]. The manipulation of row spacing, plant density, and the spatial arrangements of cotton plants, for obtaining higher yield has been attempted by agronomists for several decades in many countries. The concept of high-density cotton planting, more popularly known as Ultra Narrow Row (UNR) cotton was introduced by Briggs *et al*[60] (1967). UNR cotton has row spacing as low as 20 cm and plant population ranges from 2 to 2.5 lacs plants/ha, compared to conventional cotton where rows are 90 to 100 cm apart and have a plant population of about 100,000 plants/ha. Kranthi[61] (2012) visited Brazil, studied pure-line high-density short-season (HD-SS) grown there, and made the following observations:

- Like India, a very large area of cotton in Brazil is under rain-fed conditions and HD-SS was found perfectly suitable under these conditions.
- Compact sympodial varieties were cultivated in Brazil which was suitable for high-density planting geometry. High-density planting used the specification of 90X10 cm and 76X10 cm and 45X10 cm spacing was used in ultra-narrow-row planting.
- Higher productivity in Brazil was achieved through the development of compact monopodial (sympodial) varieties[59].

Based on this study,scientists at the Central Institute for Cotton Research (CICR), Nagpur, also developed a pure line high yielding non-Bt HD-SS rain-fed varieties of *G. hirsutum* and *G. arboreum*. The *G. hirsutum* pure line non-Bt HD-SS variety PKV-081 produced an average of 1944 kg of seed cotton/ha at 16 plants $m^{-2}$, whereas the pure line non-Bt HD-SS *G. arboreum* variety CINA-404 yielded an average of 1,973 kg/ha at 22 plants $m^{-2}$(table 1). Seed cotton yields in the two non-Bt rain-fed kinds of cotton were about half those in irrigated cotton in southern California, but they were about 2.2 times the current average yield of long season Bt hybrids in Maharashtra. In spite of the effects of the rainfall on the yields, the HD-SS have the ability to better utilize the rainfall thereby reduce the yield variability[62,63]. Equally important, the HD-SS varieties were found to escape the PBW attack, since they germinate in mid-June coinciding with the monsoon rains when adult emergence from overwintering pupae has occurred. Also, the short season length of fewer than 150 days was found unsuitable for the development of the PBW population.

Table: 1 Data on pure line non-Bt HD-SS varieties (kg seed cotton/ha): data reproduced from Venugopalan *et al*[62] (2011).

| **Plants/ha** | **Anjali** | **PKV-81** | **CCH-724** | **CNH120MB** | **NISC-50** |
|---|---|---|---|---|---|
| *G.hirsutum*- kg/ha seed cotton | | | | | |
| 55000 | 502 | 1200 | 679 | 1030 | 1056 |
| 111000 | 847 | 1714 | 843 | 976 | 890 |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
| 111000 | 853 | 1418 | 681 | 1138 | 1103 |
| 166000 | 966 | 1921 | 864 | 1250 | 1016 |
| 166000 | 796 | 1967 | 835 | 1289 | 1052 |
| Plants/ha | CINA-404 | PA-255 | AKA-07 | JK-5 | PA-08 |
| *G.arboreum*- kg/ha seed cotton |  |  |  |  |  |
| 111000 | 1430 | 1259 | 1163 | 1223 | 1090 |
| 166000 | 1550 | 1595 | 1349 | 1452 | 1318 |
| 166000 | 1610 | 1349 | 1456 | 1151 | 1455 |
| 222000 | 2173 | 1625 | 1815 | 1842 | 1509 |
| 222000 | 1772 | 1226 | 1419 | 1734 | 1479 |

NB: Plants m–2 = plants/ha/10,000.

### 3.3 Sterile insect technique (SIT)

As early as 1937, E. F. Knipling had conceived an approach to insect control in which the natural reproductive processes of the screwworm fly was disrupted by chemical or physical mechanisms, thus rendering the insect sterile[64]. Sterile insects are released into the environment in very large numbers (10 to 100 times the number of native insects) to mate with the native insects that are present in the environment. A native female that mates with a sterile male will produce infertile eggs. Since there are 10 to 100 times more sterile insects in the population than native insects, most of the crosses become sterile. As the process is repeated, the number of native insect decreases and the ratio of sterile to native insects increase, thus driving the native population to extinction[65].

A sterile moth release program was initiated in 1968 to exclude pink bollworm from cotton in the Central Valley of California[66]. The male moths were irradiated (via gamma radiation), sterilized and reared in thousands, and released periodically over cotton fields with the help of airplanes. These irradiated males would compete with the native males, thus mating with sterilized male prevents egg hatching or the produced offspring are sterile[67,68]. Van Steenwyk *et al*[69] (1979) reported that mass-reared sterilized males were less competitive than their native counterparts, while mass-reared sterilized PBW females were equal to or more competitive than native females. However, he also indicated that the combined release of both male and female PBW provided a sterile population that was as competitive as native males and females in mating ability.

A similar multi-tactic eradication program was also launched in Arizona for four years (2006-2009) to delay pink bollworm resistance to Bt cotton[70].Special emphasis was given on the number of sterile insects released and the frequency of release, thus the release rate of sterile PBW was more than 600 times higher than the simulated rate, which resulted in suppression of resistance to Bt cotton for more than 20 years without refuges. The US Environmental Protection Agency (EPA) reviewed the proposed eradication program[71] and based on the results, allowed the Arizona cotton growers to plant up to 100%

Bt cotton producing either one toxin (Cry1Ac) or two toxins (Cry1Ac and Cry2Ab). This resulted in the dramatic decline of pink bollworm populations in Arizona since the eradication program began in 2006.

With the abundant benefits that SIT can offer, it seems that this technique is perfectly relevant in the Indian context, in the management of the pink bollworm and other bollworms. But Indian conditions have many problems when it comes to the application of such techniques since the farmer holdings are very small and the application of these techniques becomes complicated. Therefore, the application of this technique requires the revision of some government policies and the assistance from the farmers.

### 3.4 **RNA interference**

The discovery of RNA interference (RNAi) constitutes an important milestone in the study of regulatory RNAs[72]. In this process, small (s)RNA molecules of 18–31 nucleotides(nt) long effectuate a sequence-specific gene silencing response, acts at the post-transcriptional level through cleavage or blockage of longer RNAs containing a matching sequence[73].The RNAi technique has been thoroughly researched in the Western corn rootworm (WCR) *D. virgifera virgifera*[74-76]. Baum *et al*[74] (2007) genetically engineered a transgenic corn crop, to express dsRNA against the V-ATPase. When insect feed on the modified plant of *D. virgifera virgifera*, larvae get stunted and premature death of the insect take place. The results were encouraging as a crop protectant due to less feeding damage[74]. Based on the fact, first RNAi-based insecticide was approved by the United States Environmental Protection Agency (EPA). This plant-incorporated protectant (PIP) employed stacking of different type of genes in a single host: dsRNA coupled with different type of Bt-proteins, also targeted the WCR Snf7 gene and was expressed in the plant[77]. The gene, Snf7 also works as a protein trafficker and when it was regulated, it resulted in mortality of the insect[78]. This strategy has very less chances of development of resistance due the diversified genes used in it[77]. In cotton crop, same study was done against the cotton bollworm *Helicoverpa armigera* where, the plant-mediated expression of dsRNA targeted the cytochrome P450 monooxygenase gene (CYP6AE14) that could increase the toxic effects of gossypol, a cotton metabolite that is otherwise tolerated by the cotton bollworm[79]. The silencing of CYP6AE14 led to delayed larval growth when gossypol was supplemented in the diet[79]. On the lines of *H. armigera,* these studies can also be conducted for PBW.

### 3.5 Chemical insecticides

Different surveys conducted by CICR In Gujrat, revealed that various chemical insecticides supported the growth of PBW population, especially the mixture of two insecticides i.e mixture of monocrotophos + acephate, when sprayed 3-4 times during early stages of the Bt-cotton crop, leads to reversal of reproductive to vegetative phase; emergence of fresh green leaves and delays maturity of the crop. Continuous application (3-4 times) of this combination results in staggered flowering and fruiting. Since flowers remain for much longer period than normal, they can attract bollworms, therefore continuous maintenance of pink bollworm inoculam takes place in such fields[18]. Therefore, use of such combination of insecticides should be avoided. Infestation of pink bollworm was high in the open bolls and green bolls of second picking in such fields. Survey conducted by CICR also revealed that quinalphos or thiodicarb, type of insecticides should be used in earlier stages and use of synthetic pyrethroids should be used after October at economic threshold levels of damage since the use of synthetic pyrethroids in earlier stages will lead to whitefly population outbreak. Wherever farmers had sprayed synthetic pyrethroids in late October or early November, pink bollworm infestation was negligible. Selection of hybrids that are sucking pest resistant also helps in control of PBW population, since it supports in avoiding the application of chemicals such as monocrotophos, acephate, thiomethoxam, acetamiprid, imidacloprid or clothianidin[80].

## 3.6 Farming practices

Cultural control plays crucial role in reducing the carryover of PBW to the next season. Therefore, essential practical measures should be taken to prevent the spread of PBW in the field, which includes pre-planting, post-harvest and off-season measures[81]. Practices such as removing of cotton stubbles after the cotton crop season, timely termination of the crop, avoid stacking of cotton stalks for fuel purpose over long periods and deep summer ploughing to expose the pupae of the surviving larvae are the major post-harvest season cultural practices. Pre-planting practices i.e selecting timely and early maturing varieties, drying seeds for 6-8 hours under sun and delinting seeds before sowing is the practice which can decrease the PBW incidence to some extent[18]. Sowing time also play key role in the incidence and extent of damage done by the pest. In north India, the sowing time range is narrow, i.e. from 15 April to May whereas south and central India have staggered sowing, varying from April (under irrigated condition) to july (under rainfed condition), thus providing continous influx of source plant for thriving of PBW[82]. Also the care should be taken that the long duration storage of raw cotton in ginning mills and market yards is avoided because that can serve as a source of pink bollworms to the ensuing crop. In central India, wherever irrigation facilities are available, farmers maintain ratoon, which can increase the PBW incidence[83].

## 3.7 Bio-control agents

Several genera of Ichneumonids, Braconidae and Trichogrammatidae found attacking PBW. Apanteles, Bracon and Chelonus are the generas of family Braconidae that have been contributed in management of PBW. Inundative release of many parasitoids was done in Arizona between the time periods of 1969-78, however the best performance was achieved by egg- larval parasitoids *Chelonus* spp. (Braconidae). Legner and Medved (1979)[84] reported that *Chelonus* sp. nr. *curvimaculatus* (Cameron) gave 69.9 % reduction in infested bolls by PBW in northwestern Australia bollworm larvae under field conditions.

Several predaceous orders attack PBW such as Dermaptera, Coleoptera, Hemiptera and Neuroptera. Predators mainly attack eggs because they exposed more as compared to larvae and pupae[85,86]. The predator belonging to dermaptera, *Labidura riparia* (Pallas) can attack all the immature stages of PBW along with pupa[86]. Coleopteran predators mainly attack early instar larvae and eggs. According to Orphanides *et al*., (1971)[86], *Chrysoperla carnea* is the only neuropteran that attack PBW in California.

Steinernematid (Rhabditida: Steinernematidae) are the nematodes which act as obligate insect parasites[87] and they are associated with a symbiotic bacteria, *Xenorhabdus* spp.[88]. The nematodes enter the insect body from the inhabitant soil and bacteria are released in insect haemocoel that cause septicemia, leading to the death of the insect[89]. The nematodes may pass through several generations, and once host reserves are depleted a new generation of infective juveniles exit the cadaver[90]. *S. carpocapsae* and *S. riobravis* are found very useful in management of diapausing PBW larvae. Entomopathogenic nematodes have positive affinity towards the other beneficial insects and do not hamper the application of most chemical fertilizers and insecticides[91,92].

### 3.8 Role of extension functionaries

Extension functionaries play crucial role in disseminating the knowledge among the farmers because they are directly connected to them. Therefore, adoption of survey and surveillance techniques, resistance monitoring studies and pest forecasting services carried out under IRM programme help farmers in making the decision regarding the pest i.e. Central Institute of Cotton research (CICR) issues weekly advisories in nine local languages and English in the CICR web site (http://www.cicr.org.in/ weekly_advisory.htmare). Very good initiative is taken by CICR where weekly advisories (E-Kapas) are sent to 11,893 farmers in Gujarat and 1,80,000 farmers across India through voice mail. CICR project staff conducts IRM campaign at various field sites (150 sites) across Gujrat. All India Coordinated Crop Improvement Project (AICRIP) on cotton also involves Front-Line demonstrations (FLDs) for farmers[18].

## Conclusion

In a country like India, where farmers are indulged in many problems like lack of resources, small farm holdings, illiteracy, poverty, a slower rate of mechanization, vague government policies, etc., they are incapable of handling such problem at their level. Therefore, it becomes important to find some serious solution to this problem and the government requires to interfere and invest in research and development (R & D) methods before it’s too late so that the situation doesn't get worse as in case of American bollworm (*H. armigera*).

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