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Review Article

Eco-friendly Management of *Pectinophora gossypiella* resistance to transgenic Bt Cotton: A Review

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ABSTRACT

Pectinophora gossypiella (Saunders) has had a crucial ecological and economic impact on transgenic cotton production in Pakistan since 2014. For effective control of this pest, a comprehensive strategy is necessary. This article provides an overview of integrated techniques to manage *P. gossypiella*. This study explores the integration of various pest control strategies, including cultural, biological, and physical approaches, all intended to lower the pink bollworm infestation in an environmentally friendly manner. Cultural practices such as changing crop varieties with their sowing time, removing infested plant remnants (leftover bolls), and stacking vertically interrupt the *P. gossypiella* life cycle. Biological control agents like coleopteran, dipteran, etc serve to limit the *P. gossypiella* population naturally. The application of sex pheromones (trap) and PB ropes aids in maximizing the crop yield by reducing the pink bollworm infestation. The use of proper refuge aids increased the crop yield as well as maintain the sustainability of transgenic cotton, like in China (hybrid cultivation). Sterile insect techniques successfully eradicate the *P. gossypiella* population, like in the United States. These integrated pest management (IPM) strategies have resulted in significant reductions in PBW infestation and pesticide usage, as well as improvements in yield, economic benefits, and environmental quality. For area-wide success, stakeholder cooperation and active engagement are essential, and outreach has been utilized to increase public awareness.

Keywords: Biological control, Cultural Control, Pink bollworm, PB rope, Sex Pheromone, Sterile Insect Techniques, Refuge.

INTRODUCTION

Cotton (*Gossypium hirsutum* L) is well-known as “the King of natural fiber” as well as “White Gold” worldwide (Ismail, 2021), and is one of the main cash crops of Pakistan. Among the different cotton-growing countries, Pakistan ranks in the 7th position (Statista, 2023). Cotton shares in the country's GDP are 0.7% and 2.7% in GDP and value addition, respectively (Economic Survey of Pakistan, 2023). Abiotic variables like salinity, drought, and high temperatures reduce the cotton yields as well as numerous insect pests cause cotton yield losses including sucking (jassid, whitefly, thrips, mites, etc.) and chewing (bollworms and weevils) insect pests. Among bollworms, *Pectinophora gossypiella* S. (Lepidoptera: Gelechiidae) is the most damaging cotton pest and causes losses of millions of bales globally. It reduces almost 59% of normal boll opening, 62% of seed cotton yield losses, and 47% of oil contents losses (Patil, 2003). It was also reported that cotton bales of one million worth approximately 14 billion, are affected each year by P. gossypiella (Pink bollworm) in Pakistan (Anonymus, 2019). Similarly, many other studies reported that it causes severe cotton yield reduction in Pakistan (Abro et al., 2003) due to

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that it causes severe cotton yield reduction in Pakistan (Abro et al., 2003) due to infestation at different growing stages (i.e. flowers, squares, green bolls, and open bolls) (Ashfaq et al., 2006).

Globally, PBW is the most damaging pest of cotton bolls. Larvae of PBW cause more economic damage (yield loss) to cotton than adults (Figure 1). It hatches from eggs, and quickly eats flower stamens, squares, and bolls (especially 10 to 24 days old) resulting in failure of the opening of bolls, shedding of bolls lint damage, and speed loss. Pink bollworm damaged bolls are at risk of secondary bacterial infection that causes the boll rind to blacken on the exterior. The exit of PBW larvae can be revealed by a small hole (1.5 to 2 mm diameter) from a green boll (Henneberry and Naranjo, 1998; Hutchison, 1999).

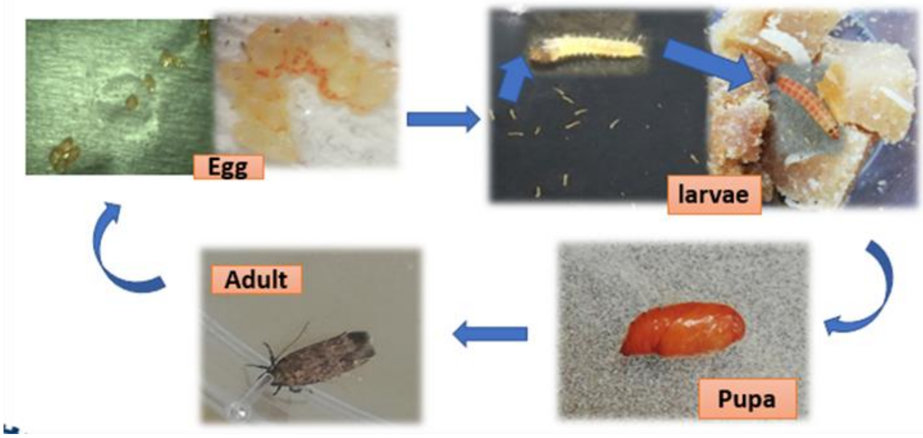

Figure 1. Life cycle of Pink bollworm (with normal and microscopic view of egg and neonates).

Many farmers spend 80% of their total pesticide on cotton to control the bollworm management (Arshad and Suhail, 2011). Due to the intensive use of pesticides, bollworms have started to resist insecticides (Forrester et al., 1993) which is mainly due to selection pressure that results in the onsets of resistance. To resolve the problem of insecticide resistance, transgenic Bt cotton was developed with the soil-dwelling bacterium Bacillus thuringiensis, a safe, effective, and specific insect management tool. This crop produces a lethal toxin (Cry1Ac protein) to manage lepidopteran pests (Shelton et al., 2002; Wu and Guo, 2004; Mendelsohn et al., 2003; Shelton et al., 2002; Wu and Guo, 2004). In 1996, the first transgenic cotton was cultivated in the USA and then in other countries including China, Australia, Argentina, Mexico, Colombia, India, South Africa, Brazil, (James, 2006), and Pakistan in 2010.

Transgenic Bt cotton has increased the farmer benefits by decreasing conventional pesticide use (Abedullah et al., 2015; Godfray et al., 2010; Lu et al., 2012; Romeis et al., 2019). Because of intensive and area-wide cultivation of transgenic cotton, bollworms developed resistance against Bt cotton (Kranthi et al., 2006; Tabashnik et al., 2008). Recently pink bollworm has become a severe risk to transgenic Bt cotton because of its monophagous nature (only feed on cotton). Due to area-wide cultivation of transgenic cotton, Pink bollworm start to resist this because of selection pressure in its population (Shelton et al., 2000; Huang et al., 2006). Resistance may evolve as a consequence of behavioral changes, detoxification, and maturation processes (Onstad and Knolhoff, 2023).

Pink bollworm resistance was assessed against Cry1Ac in the USA in 1997 (Liu et al., 1999; Patin et al., 1999; Tabashnik et al., 2000), also in China from 2005-2007 to 2008–2010 (Wan et al., 2012) as well as in India (Dhurua and Gujar, 2011; Naik, Kumbhare, et al., 2018). In the USA and China, pink bollworm (PBW) have evolved resistance in the laboratory strains (Tabashnik et al., 2009; Wan et al., 2012, 2017) than in India, where it has developed practical resistance in the field against Cry1Ac and Cry1Ac + Cry2Ab (Fabrick et al., 2014; Mohan et al., 2016; Naik, Kumbhare, et al., 2018; Ojha et al., 2014). While in Australia, resistance was reported in Helicoverpa armigera against Cry1Ac (Gould, 1998; Olsen et al., 2005). In Pakistan, pink bollworm infestation of cotton has been reported against Bt Cotton cultivars producing Cry1Ac but resistance has not been reported yet (Abbas et al., 2016; Akhtar et al., 2016). In Pakistan, the survival ratio of *H. armigera* on Bt cotton was observed less unlike non-Bt cotton (Ullah et al., 2014). Similarly, in another study, H. armigera exhibited resistance surging against Cry protein Bt cotton (containing Cry1Ac single gene) in Pakistan (Ahmad et al., 2019).

Pink bollworm populations persist on Bt cotton, despite initial control efforts. Bollgard II, a cotton transgenic plant, failed to manage the infestation, causing crop damage and impacting the cotton trade chain. This has led to reduced market prices and concerns among scientists and the cotton industry. There are many reasons behind insect resistance against transgenic cotton: one is that insects have evolved resistance and another is that the level of resistance in plants is insufficient to control the pest.

Insect resistance against transgenic cotton

Firstly, it was reported that insect pests had developed resistance against transgenic crops (Tabashnik and Carrière, 2017) as presented in Table 1. The binding of Bt toxins to the insect target site (midgut apical membrane) is important for the death of pest larvae as shown in figure 2 (Adang et al., 2014; Fabrick et al., 2023), but the reduction of this binding causes the resistance of pests to toxins (Tabashnik, 2015; Zhang et al., 2012). Resistance to this pest is due to mutation in the cadherin gene of PBW. Resistance in Bollworm evolution to Bt toxin (Cry) is associated with the mutation of four types of midgut receptor proteins: cadherin, aminopeptidase N, ATP-binding cassette (ABC) transporters, and alkaline phosphatase (Jurat-Fuentes and Crickmore, 2017; Pardo-López et al., 2013; Wu, 2014; Xiao and Wu, 2019). Pink bollworm develop resistance against Bt cotton (Cry1Ac) evaluated in Arizona field strains under laboratory conditions (Tabashnik et al., 2002). Sixteen resistance alleles (PgCad1) of pink bollworm against Cry1Ac been documented, four strains from laboratory selected population in China, four strains (r1-r4) from laboratory selected population of Arizona (Fabrick and Tabashnik, 2012), eight strains (r5-r12) from India field strains (Fabrick et al., 2014). Its resistance was reported in the field sample of PBW from Gujrat, India in 2010 and 2011 (Bagla, 2010; Dhurua and Gujar, 2011; Fabrick et al., 2014). However, this resistance is due to improper management practices as not using the proper refuge system.

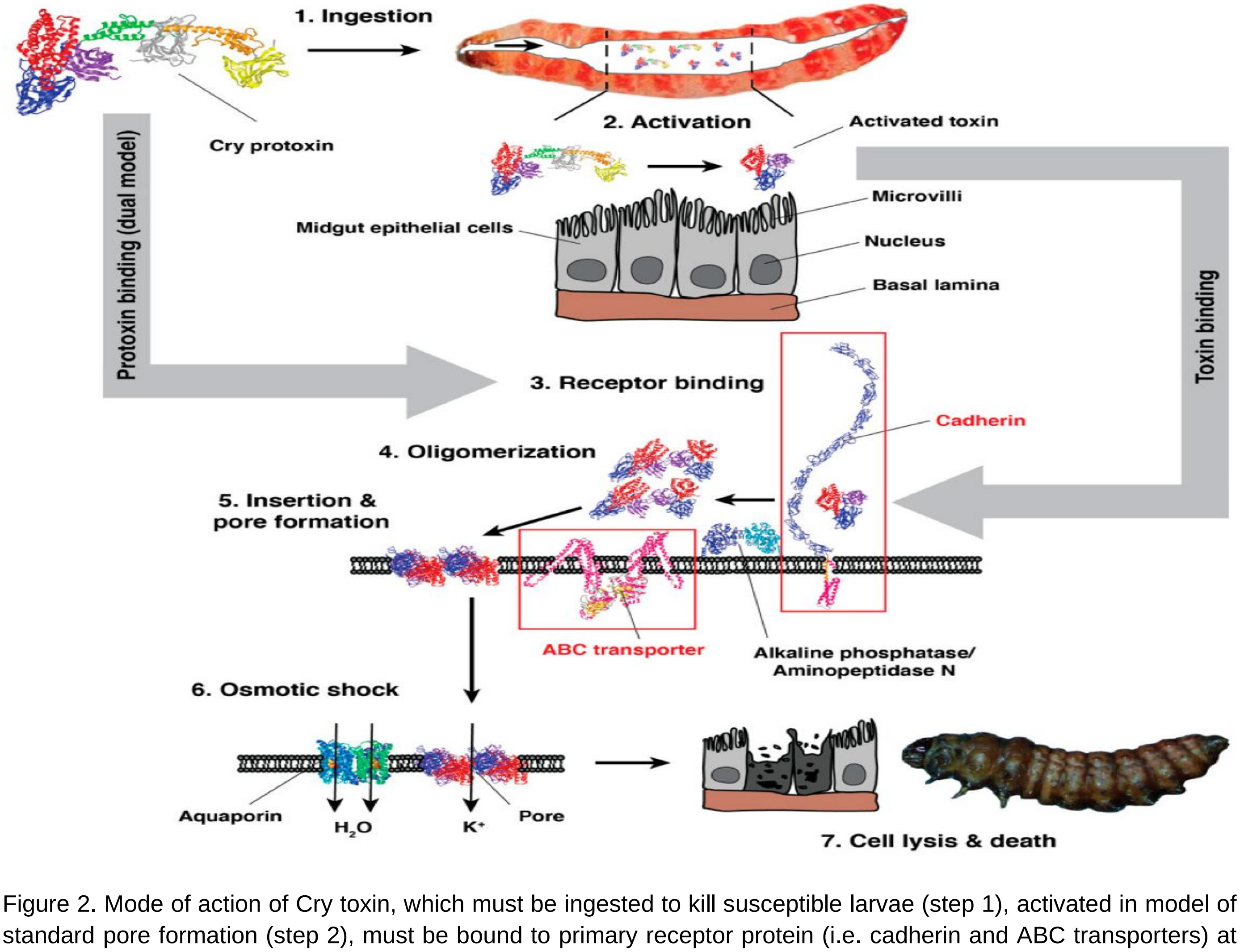

Figure 2. Mode of action of Cry toxin, which must be ingested to kill susceptible larvae (step 1), activated in model of standard pore formation (step 2), must be bound to primary receptor protein (i.e. cadherin and ABC transporters) at midgut epithelium surface (step 3), then oligomerizes (step 4), and interact with co-receptors (as alkaline phosphate or aminopeptidase). Next, these oligomers enter into pores producing epithelial membrane (step 5), these pores induce osmotic shocks in cells of the midgut by fast cation influx (step 6), then these cells swell or enlarge due to water uptake via aquaporin water channel proteins and finally lyse. Ultimately, the insect dies from acute injury, starvation,

and septicaemia. Another dual model suggests that both protoxins and activated toxins attach to midgut receptors and produce toxicity via a second process (Fabrick et al., 2023).

Gene-pyramided crops were engineered to produce two or more insecticidal proteins (i.e. Cry or Vip) to delay this resistance evolution. Double gene transgenic cotton procedure double toxin either Cry1Ac and Cry1F or Cry1Ac and Cry2Ab used to control the resistance against single gene (Cry1Ac) in the USA, China, Australia, and India, (Brévault et al., 2013; Downes and Mahon, 2012). Double gene cotton (Bollgard II) was found effective for controlling the target insect pest of cotton (Kranthi et al., 2005). It was found that gene-pyramided plants were found more effective in delaying insect resistance than single toxin plants by using a simulation model (Zhao et al., 2003; Roush, 1998; Zhao et al., 2003). This model also assumes that both proteins (i.e. Cry1Ac and Cry2Ab toxins) production remains constant and at a level to control the target insect all over the growing season. The addition of Cry2Ab with the Cry1Ac gene has been providing synergistic effects against cotton bollworms and helps to manage resistance development (Wei et al., 2015). Gene pyramiding of Vip3AcAa and Cry1Ac increased the mortality of larvae of *A. ipsilon, S. litura*, and *S. exigua* than single-gene Bt (Cry1Ac) cotton (Chen et al., 2017). Pyramids finds effective when each insecticidal toxin kills susceptible target insects (99.75%) and acts independently (Carrière et al., 2015). Gene pyramiding helps to delay the resistance against single toxin Bt cotton (Roush, 1998; Zhao et al., 2003).

While asymmetrical resistance was also observed in Pink bollworms against the Cry1Ac, and the Cry2Ab (Tabashnik et al., 2009). In India, Pink bollworms become resistant to the Cry2Ab which is associated with a mutation in the protein PgABCA2 (ATB-binding cassette transporter gene) (Mathew et al., 2018). In Amreli and Bhavnagar districts (Gujrat incidence) of India, the incidence of PBW was found 40-80% in BG II cotton hybrid (Kranthi, 2015b). A study reported that pink bollworm adopts resistance against Bt cotton containing double genes in India (Naik, Kumbhare, et al., 2018). Multi-toxin resistance permits the survival of Pink bollworms on gene pyramiding Bt cotton having genes Cry1Ac and Cry2Ab (Fabrick et al., 2015).

PBW Resistance against Bt toxin Cry1Ac (single-gene) due to mutation of cadherin gene (Carrière et al., 2006; Fabrick et al., 2014; Morin et al., 2003) and Bt toxin Cry2Ab (double gene) due to ATP-binding cassette transporters (ABC protein) (Mathew et al., 2018). Four recessive alleles of cadherin (mutation) caused resistance in PBW against Bt protein (Cry1Ac) in the laboratory (Carrière et al., 2006; Fabrick et al., 2014; Morin et al., 2003; Tabashnik et al., 2005; Tabashnik, Liu, et al., 2004). Bollworm was reared in the laboratory for 44 generations and observed resistance against Bt toxin was increased from106 fold than susceptible strain (Wu and Guo, 2004). A novel allele (r16) of PgCad1 (cadherin gene) was linked with resistance in PBW against Cry1Ac toxin (Wang et al., 2019). Inheritance of resistance against Cry1Ac was recessive, associated with this allele and cadherin gene observed on BBMVs (brush border membrane vesicles) in susceptible larvae (midgut) than resistant larvae. It was also concluded that a homozygous strain for r16 had a resistance of 300 fold against Cry1Ac, and 2.6 fold against Cry2Ab.

Table 1. Resistance of Pink bollworm to Bt cotton (Cry1Ac and Cry1Ac + Cry2Ab toxins).

Insect	Toxin	Country	First-Year[a]	Resistance	Condition	Citation
P. gossypiella	Cry1Ac	USA	1996	2002	Lab	(Liu et al., 1999; Patin et al., 1999; Tabashnik et al., 2000; B. E. Tabashnik et al., 2005)
P. gossypiella	Cry1Ac + Cry2Ab	USA	2003	2009	Lab	(Brévault et al., 2013; Fabrick et al., 2015; Tabashnik et al., 2009)
P. gossypiella	Cry1Ac	China	2000	2005	Lab	(Tabashnik et al., 2012; Wan et al., 2012)
P. gossypiella	Cry1Ac	India	2002	2009	Field	(Dhurua and Gujar, 2011; Naik, Kumbhare, et al., 2018)
P. gossypiella	Cry1Ac + Cry2Ab	India	2006	2014-2015	Field	(Kranthi, 2015a; Naik, Kumbhare, et al., 2018)
P. gossypiella	Cry1Ac	Pakistan	2010	2014	Field	(Abbas et al., 2016; Akhtar et al., 2016; Hassan et al., 2021; Khuhro et al., 2015)
P. gossypiella	Cry1Ac + Cry2A	Pakistan	2016	2020	Field	(Hanif et al., 2023; Hanif, Saeed, Ali, Ishtiaq, Khan, et al., 2024)

[a] First year of commercial planting of a Bt crop in the region was monitored.

Expression of Transgenic Protein in Transgenic Cotton

Expression of Cry1 toxin in Bt Cotton varies over the growing season during field conditions because of variations in environmental factors including temperature, moisture, drought, and insect infestation (Mahon, et al., 2002) and agronomic factors such as variety, growth stage, plant age, tissues, and species, etc (Abel et al., 2004; Adamczyk, Hardee, et al., 2001; Wan et al., 2005). It was found that the Cry1Ac toxin concentration is reduced during the crop growing season whereas the Cry2Ab toxin level remains the same (Adamczyk, Adams, et al., 2001; Carrière et al., 2010; Showalter et al., 2009). Bt expression was also decreased continuously with plant age (Adamczyk et al., 2009; Adamczyk and Sumerford, 2001; Kranthi et al., 2005; Poongothai et al., 2010; Wan et al., 2005). This decrease in the expression of Cry1Ac has been linked to a reduction in mRNA production of a target gene and promoter methylation (35S) in the plant during later growth stages (Adamczyk et al., 2009). In a research study, inter and intra-variations of toxin (Cry2Ab) expression were observed in plants and the level of the toxin was found higher in leaves than in boll rind (Kranthi et al., 2005). This shows that toxin levels decrease with plant age and higher toxins are found in leaves than in fruiting parts (Bakhsh, Rao, et al., 2012; Chen et al., 2000; Kranthi et al., 2005; Manjunatha et al., 2009).

During the vegetative stage, Cry1Ac toxins were found more in leaves as compared to the reproductive stage (Fitt, 1998; Greenplate et al., 1998; Olsen and Daly, 2000; Xia and Guo., 2004). In another study, the same results observed that leaves have higher expression than fruiting parts (Abel et al., 2004; Adamczyk and Sumerford, 2001; Bakhsh et al., 2010; Bakhsh, Siddique, et al., 2012; Greenplate et al., 2000; Greenplate et al., 1998). It was reported that after sowing, the Cry1Ac level was reduced below the toxic level (1.9 ug/g) within 110 days (Kranthi et al., 2005). He also determined that toxin levels were higher in squares than in bolls and flowers. According to him, toxin levels are lower in flower ovaries and the rind/peel of green boll is a favorable site for the attack of bollworm. It is also reported that during the seedling stage, the toxin level of Cry1Ac was higher in leaves as compared to other parts (stems, roots, and petioles) while during the flowering stage, ovaries (at the time of anthesis) express higher toxin than other parts such as stamens and pistils (Chen et al., 2000).

Bt toxin production is highly variable and it is not only varied by different cultivars (Cheema et al., 2016) but also varies within plant parts and tissues (Khan et al., 2018). As the plant matures, levels of Cry1Ac toxin and protein content are reduced (Holt, 1998). The environmental effect on the effectiveness of Bt cotton is unforeseen because secondary product levels increase during physical damage and physiological stress (Dixon and Paiva., 1995) which disturbs the ability of Bt toxin (Arteel and Lindroth, 1992; Navon et al., 1993). Due to the reduction of insecticidal protein expression level, loss of efficacy of insect-resistant of plants was associated (Benedict et al., 1996; Finnegan, 1994). The expression of Cry1Ac in Bt cultivars throughout the entire season has shown that with the plant ages, the endotoxin level of Cry1Ac decreases (Adamczyk and Sumerford, 2001; J. Greenplate et al., 2000; Sachs et al., 1998; Zaman et al., 2015).

Moisture and temperature affect the developmental process of cotton, and its physiological metabolisms (Reddy et al., 1992; Reddy et al., 1991). Chen et al., (2012) reported that the insecticidal (Cry1Ac) protein expression of the leaf was greatly affected at the peak of the flowering stage (due to high temperature) as well as and also affected during the boll filling stage (due to both relative humidity and extreme temperature). The expression of Bt toxin is affected by low and high temperatures (Chen et al., 2005; Chen et al., 2000; Xia and Guo., 2004). Under highest humidity caused less decline of Bt toxin at the boll developing period and the lowest humidity declined the leaf Bt toxin protein levels under the highest temperature (Yuan et al., 2012) at greenhouse conditions. The drought stress also lessened the protein expression level in transgenic cotton (Wang et al., 2001).

Toxin expression was also decreased by nitrogen deficiency or elevated CO2 (Bruns and Abel, 2003; Chen et al., 2005; Coviella et al., 2002). It is also dependent on water stress (Traore et al., 2000). Soil water shortage reduced the Cry1Ac toxin expression in bolls (Zhang et al., 2017). The level of Bt protein/toxin was greater in cotton plants sowing in early May than those cotton sowing in early April (Pettigrew and Adamczyk, 2006). The effect of potassium, phosphorous, and rainfall on the expression of the toxin was more accessed than the effect of irrigation (Huang et al., 2014). Saline or drought soil conditions affect the Bt protein expression (Rochester, 2006). Habitats stressed with low, medium, and high salinity declined in the expression of the toxin than normal habitats (Luo et al., 2017).

In insect pests, both Cry1Ac and Cry2Ab genes show different types of binding in the epithelial membrane of the target insect (Morse et al., 2001). This binding with midgut receptors, aids in the pores formation that leads to the death of target insects but the pore formed by Cry1Ac is changed than the pores formed due to Cry2Ab (Akhurst et al., 2009; Roush, 1998). It is reported that resistant strains of H. armigera against Cry1Ac were observed as susceptible in contrast to Cry2Ab, hence there is no contribution of cross-resistance (Akhurst et al., 2009; Knowles and Dow, 1993).

Expression of Cry1Ac declines because of methylation of the promoter or post-translational gene silencing during late stages (Xia and Guo., 2004) whereas Cry2Ab displays 10-fold greater expression than Cry1Ac (Greenplate et al., 2000).

INTEGRATED PEST MANAGEMENT OF PINK BOLLWORM

The Pink Bollworm, an interior feeder, poses a significant challenge to insecticide control, necessitating the implementation of integrated pest management strategies. In India and Pakistan, where Bt cotton is no longer reliable for managing pink bollworm populations, integrated pest management (IPM) is required. Pink bollworm IPM tactics include cultural control, biological control,mass trapping, and mating disruption using pheromones as an early strategy rather than chemical control. Integrated Pest Management (IPM) is an environmentally friendly pest management method that eliminates the need for chemical pesticides while minimizing pollution and maintaining biodiversity. IPM promotes targeted control measures, which assist in preventing insect resistance, saving natural resources, and encouraging sustainable agriculture. It also decreases pesticide exposure for humans and wildlife, which helps to maintain long-term environmental and ecological health. A study in Nagpur, Maharashtra, found that 558.44% of cotton growers had medium knowledge about integrated pest management practices, while the majority (60.83%) had moderate adoption of these practices (Mane et al., 2019). There are following tactics have been proven successful in controlling pink bollworm:

Cultural and off-season control practices

Cultural practices that affect pink bollworm survival have been reducing the overwintering populations. The cultural control recommendation for the season includes the destruction of pink bollworm pupae during the season through frequent hoeing and soil cultivation, followed by heavy irrigations. Cotton planting and harvesting should be done uniformly within the time ranges provided by local Agricultural Extension Services (Staten et al., 1993). Plant growth regulars should be used to terminate crops quickly because late-maturing crops are attacked heavily (50–75%) by bolls that display damaged locules in open bolls. The crop should not receive its final irrigation after September 30th in order to prevent late fruiting and boll production.

Other cultural techniques, such as timely exfoliation, stalk destruction, off-season irrigation, and crop waste burial by routine tillage, will also help to lower diapausing populations during the off-season months (Staten et al., 1993). Researchers have found that areas that have been ploughed and shredded have shown a greater than 80% decrease in moth emergence (Adkisson et al., 1960). Diapausing larvae spend winter in immature cotton bolls, soil, and garbage (Bariola, 1984). Removing late-season immature cotton bolls is an effective way to limit overwintering pink bollworm populations (Kittock et al., 1973). If cotton stalks were placed horizontally, 3.5% of the larvae survived in the lower half of the heap, whereas O% survival when stalks were placed vertically (Attique et al., 2001).

The collection of pink bollworm (P. gossypiella) larvae from cotton wood debris is an important step in pest management. Cultural control methods such as timely sowing (Variya et al., 2023), modifications in sowing practices (Ingole et al., 2019; Javaid, 1995) crop termination (Watson et al., 1978), removal of leftover (immature) cotton bolls at end of season (Kittock et al., 1973), shredding stalks, ploughing, disking, and winter irrigation have been demonstrated to effectively kill diapausing larvae in bolls, garbage, and soil. To protect hibernating pink bollworm larvae/pupae, cultivate cleanly and remove agricultural leftovers (e.g. fallen leaves, and twigs) before the season begins, and plough deeply to expose them to the sun (Watson, 1980). Winter irrigation can significantly reduce pink bollworm populations by 50-70%, with December flooding being more effective than November or January. Bt cotton can also help prevent pink bollworm damage. According to Novo and Gabriel, (1994), cotton seed mills with suitable fans for removing gin debris (carrier of overwinter bollworm) have less cotton bollworm compared to mills without such equipment.

Controlling pink bollworm in the field is unavoidable after careful monitoring, with chemical control being the last resort when the population exceeds the economic threshold. Integrated management programs should be implemented during the cotton season to maintain sustainability. India's IPM strategies for pink bollworm include planting early-maturing cotton hybrids, terminating crops early, avoiding ratoon cotton, deep tillage, using insecticides based on scouting and thresholds, crop rotation, biological control, and mass trapping and mating disruption using pheromones (Kranthi, 2015b; Mohan, 2017).

Biological Control

Natural insects prey on insect pests, including predaceous Hemiptera, Coleoptera, and Neuroptera and Dermaptera. Nematodes and B. thuringiensis have been utilized as control agents in various studies (Ahmad et al., 2011; Gouge et al., 1999; Henneberry and Naranjo, 1998; Sarwar, 2013). Several research studies demonstrated the efficacy of frequent natural enemies (Predator and parasitoids) against pink bollworm (Table 2). Pink bollworm eggs are more vulnerable to attack than other life stages due to their exposed location.

Implementing biological control in Integrated Pest Management (IPM) comes with challenges such as environmental factors, which can affect parasitoid effectiveness, and the high cost and scalability of large-scale adoption. Additionally, proper education and training are crucial to ensure farmers successfully integrate these methods. However, by overcoming these challenges, farmers can effectively reduce chemical pesticide use, promote ecological balance, and achieve sustainable pest management.

Table 2. List of natural enemies of pink bollworm.

Technical Name	Feeding Stage	Citation
Predators		
Chrysoperla carnea	Egg, 1st instar Larva and Pupa	(Orphanides et al., 1971; Henneberry and Clayton, 1985)
Geocoris punctipes	Egg, and 1st instar larva	(Hagler and Naranjo, 1994; Orphanides et al., 1971)
Hippodamia convergens	Egg, and 1st instar larva	(Hagler and Naranjo, 1994; Henneberry and Clayton, 1985; Orphanides et al., 1971)
Labidura riparia	immature stages	(Orphanides et al., 1971; Sarwar, 2017)
Pardosa milvina	Adult	(Sarwar, 2017)
Nabis americoferus	Egg	(Orphanides et al., 1971; Henneberry and Clayton, 1985)
Sinea confusa	Egg	(Henneberry and Clayton, 1985)
Orius tristicolor	Egg	(Henneberry and Clayton, 1985; Orphanides et al., 1971)
Collops vittatus	Eggs	(Henneberry and Clayton, 1985; Hagler and Naranjo, 1994)
Calosoma affine	Egg and Larval	(Orphanides et al., 1971)
Clanoptilus marginellus	Egg and Larval	(Orphanides et al., 1971)
Sinea diadima	Egg and Larval	(Orphanides et al., 1971)
Zelus renardii	Egg and Larval	(Orphanides et al., 1971)
Trachelus sp	Larval	(Orphanides et al., 1971)
Metaphidippus sp	Larval	(Orphanides et al., 1971)
Metacyrbra californica	Larval	(Orphanides et al., 1971)
Parasitoids		
Trichogrammatoidea bactrae	Egg	(Ahmad et al., 2011; El-Hafez and Nada, 2000; Gouge et al., 1999; Hutchison et al., 1990; Khanzada et al., 2016; Naranjo, 1993; Rao et al., 2024)
Chelonus blackburni	egg and larval	(Jackson et al., 1979)
Trichogramma evinces	egg	(Saad et al., 2012)
Bracon kirkpatricki	egg and larval	(Bryan et al., 1971)
Apanteles oenone	larval	(White et al., 2013)
Bracon Chelonus	larval	(Sarwar, 2017)
Bracon hebetor	larval	(Naik et al., 2023)
Apanteles angaleti	larval	(Singh et al., 1988)
Bracon lefroyi	larval	(Naik, Kranthi, et al., 2018)

Refuge

Transgenic cotton failed because of not following the cultivation rules given by Monsanto i.e. 1) sowing of non-Bt cotton 20% or more and use of insecticidal spray than microbial spray 2) sowing of 5% non-Bt cotton without the use of any microbial spray. Due to a lack of refuge plants and area-wide cultivation of Bt cotton can cause selection pressure on bollworms, potentially leading to the development of toxin tolerance or the evolution of insect resistance against Bt cotton (Huang et al., 2011). The proper use of refuge not only minimizes dependency on the insecticide but also reduces the selection pressure on target insects (Huang et al., 2006). Several tactics have been used to delay the resistance in the United States, China, and Australia but the refuge tactic is the primary approach. Insect Resistant Management (IRM) refuge strategy aids in successfully delaying insect resistance (Wang et al., 2008). The refuge is the cultivation of non-Bt cotton with Bt cotton as a refuge to reduce the selection pressure on target insect pests and this strategy increased the life period of transgenic cotton (Huang et al., 2006). Refuges crops produce an abundance of susceptible individuals that scatter and mate with resistant survivors of the Bt field (Gould, 1998; Hagerty et al., 2005; Tabashnik et al., 2005; Tabashnik et al., 2008; Tabashnik, Gould, et al., 2004).

In the USA, cultivation of non-Bt cotton was recommended near transgenic (Bt) cotton fields or in blocks as a refuge strategy to increase the durability of Bt cotton against target insects (Matten and Reynolds, 2003). Indian Committee of Genetic Engineering (GEAC) permitted the cultivation of non-Bt cotton in rows or 20% of the total cultivated area in 2002 to delay the resistance, but farmers did not grow non-Bt cotton (Stone, 2004) the Indian Government recommended that seed companies provide a non-Bt cotton bag (120 g) with every packet of Bt cotton seed (450 g) (Mohan, 2018). Then the use of a seed mixture (5–10% non-Bt seeds) was also recommended to delay resistance development (Alyokhin, 2011; Carrière et al., 2016; Onstad et al., 2011; Tabashnik and Gould, 2012).

In China, a hybrid seed mixture provides successfully delays the resistance because it contains non-Bt cotton as 25% refuge (Wan et al., 2012, 2017). In Australia, non-Bt cotton is 30% approved as a refuge (Gould et al., 1997; Wilson et al., 2018). In Pakistan, non-Bt cotton is recommended as 10% (Agriculture Department Government of Punjab, 2020) but farmers have sown 100% Bt cotton. To test the strip row structure of refugia with different levels/percentages from 10% to 50% by using two varieties i.e. single-gene Bt crop (JKCH1947Bt) and double gene Bt crop (MRC7017BGII). They concluded that the refuge of 30% non-Bt in single gene crops and 20% non-Bt in double gene Bt crops did not impact cotton yield than 100% Bt cotton (Gujar et al., 2010). A simulation model was used for testing of refuge from 20% to 50% (Garcia et al., 2016). They concluded that strip configuration was a highly effective tool for delaying the onset of resistance because strips provide a large range border to aid crossing between susceptible and resistant insects.

For insect-resistant management against western corn rootworm, the Use of row strip, block, or mostly seed blends with 5-20% refuge is recommended (United States Environmental Protection Agency ensp, 2010). More research work was done by following the different structures of refuge, i.e. block (Alexander, 2007), block or strips (Carroll et al., 2013), and seed mixture (Carroll et al., 2012, 2013). The research was executed to examine the different levels of refuge (i.e. 5%–50%) by using a population-based model. It was found out that a larger refuge size results in lowering the resistant population (Cerda and Wright, 2004). Similarly, more research work findings (by using simulation models) show that resistance evolution in bollworms can be significantly delayed by using refuges (Carrière and Tabashnik, 2001; B. E. Tabashnik et al., 2003).

More use of refuge aids to increase the susceptible populations from non-Bt crops which assist in dilution of the resistant population surviving from Bt cotton. Because random mating of recessive resistant (RR) individual (from Bt crop) and susceptible (SS) individual (from non-Bt crop) results in the production of heterozygous (RS) progeny (Crowder and Carrière, 2009; Huang et al., 2010; Sisterson et al., 2005; Tabashnik and Carrière, 2017). Its heterozygous progeny was killed when feeding on the Bt crop (Tabashnik and Carrière, 2017). However, refuge delayed resistance against Bt crops in bollworm (Jin et al., 2015; Tabashnik et al., 2008) (Jin et al., 2015; Tabashnik et al., 2008) and helped Bt crops to sustain for a long period (Carrière et al., 2016). So for the success of the Bt crop implementation, the refuge tactic is considered very important (Bates et al., 2005; Tabashnik et al., 2003). The farmers' long-term compliance is crucial for the refuge method to be successful (Carrière et al., 2005). To forecast pink bollworm resistance development in Arizona, the researchers used mathematical models that included experimental results on refuge percentage, resistance inheritance, fitness costs, and incomplete resistance (Tabashnik et al., 2005). In Pakistan, a research study was conducted by using the cultivation of refuge (20% non-Bt) in various layouts with transgenic cotton, and found that refugia treatment led to lower P. gossypiella infection, with maximum yield than treatment without refugia, emphasizing the importance of refuges in insect/Bt agricultural systems (Hanif, et al., 2024).

Monitoring, Mating Disruption and Mass trapping by Pheromone

Pheromones, scents released by female insects to attract males, are artificially synthesized and used in traps to monitor infestation levels. They can also be used in mass trapping and mating, and the best control method of pink bollworm. Hummel et al., (1973) named the pink bollworm sex pheromone "gossyplure" after identifying it as a combination of Z,Z- and Z,E-isomers of 7,11-hexadecadienyl acetate. Shorey et al. (1976) studied mating disruption using gossyplure in cotton fields to combat PBW. Areawide applications of PBW pheromone in California's Imperial Valley resulted in reduced pesticide use and significant yield improvements (Staten et al. 1983). Pink bollworm delta traps are used for detection, baited with rubber septa containing 4 mg of gossyplure and attached to wooden stakes around cotton fields. Traps placed at planting (1 trap per ten acre), and inspected weekly until defoliation and harvest (Leggett et al. 1994).

Synthetic pheromones, specifically gossyplure, have been widely used to detect and control *P. gossypiella*, resulting in a 60-80% reduction in the pest population (Gao et al., 1992). *P. gossypiella* infestation in cotton crops can be effectively controlled using an IPM strategy, such as pheromone traps (Kranthi 2015; Mohan 2017). Studies have shown that light traps, delta traps, and Pb rope traps significantly reduce its infestation. Sex pheromone traps have shown better results than other treatments, including novel insecticides (Nadeem et al. 2023). In India, pink bollworm mating disruption technology (SPLAT-PBW) has shown increased yields, less damage to rosette flowers, and reduced pesticide usage. These findings highlight the advantages of non-chemical insect pest control in cotton, offering an alternative to chemical pesticides in managing the Pink bollworm problem (Sreenivas et al. 2021).

Researchers have proven the effectiveness of pheromone traps like sleeve traps and yellow funnels in managing pink bollworm (Sandhya et al., 2010). Mass trapping and increased concentrations of pheromones can also be used for confused mating. Maruti et al. (2020) verified pink bollworm management through mass trapping, indicating the adaptability of an integrated pest management module with cotton, resulting in lower pest infestation and higher seed cotton yield. Rao et al., (2023) evaluated an integrated pest management approach for Pink Bollworm (PBW) in cotton in the Anantapur district of Andhra Pradesh for three years (2019-2022). The approach involved installing PBW pheromone traps, releasing *Trichogrammatoidea bactrae* weekly, and spraying Quinalphos 20 EC with water if infested bolls with live larvae were observed. Results showed that IPM fields registered significantly less rosette flower damage than farmer's practice (FP) fields, indicating the suitability of IPM components. The study found that IPM was more effective in controlling PBW in cotton.

PB Rope

Pink bollworm (PBW) populations are reduced in cotton farms using PB ropes, also known as synthetic sex pheromone ropes. PB-ropes, which release sex pheromones at high rates over a specific period, effectively reduce the number of moths caught in traps (Flint et al., 1985; Staten et al., 1987). PB rope dispersers reduce insecticide use by 37-42% in cotton fields (Staten et al., 1987). In 1992 and 1993, a study in central Greece investigated the impact of mating disruption on pink bollworm population densities and damage percentages. Their results showed reduced moth catches in treated fields, allowing successful mid-season dispenser installation (Lykouressis et al., 2004). In a research study the PB-Rope* is used in early-planted cotton fields, high moth catches fields, and sensitive sites. Dispensers are hand-twisted around the main stem of the cotton plant, near six true leaf growths before the pinhead square. The PB-Rope* dispensers are applied at 200 dispensers per acre (Staten et al., 1993). Another study found that PB rope plots yielded more than control plots, with less damage to leftover bolls at cotton stick harvest time (Qureshi et al., 2020; Sohi et al., 1999). Sohi et al., (1999) reported one dispenser discharged pheromone at the rate of 1.5 mg per day, which decreased to 0.80 mg per day after 90 days of application. Papa et al., (2000) detected release period of pheromone from dispenser was 120 days.

In India, the use of PB ropes to disrupt mating was found effective in managing pink bollworm (Patil, 2003). Radhika and Reddy, (2006) showed that, in contrast to PB ropes plots, control plots had lower cotton yields but higher damage percentages of green bolls and leftover bolls. Similarly, another research study found that PB Rope (@ 685 PB Rope L at 50 days after sowing) effectively suppressed moth catches, reduced damage percentage, and improved average seed cotton yield/ha in India's cotton fields compared to control fields (Meena et al., 2021).

In Pakistan, a research study was conducted to find the efficiency of PB-Ropes by using two applications of it (Qureshi et al., 2020). They found that PB rope remains effective for 90 days after application, and moth catches reduced in two applications of PB rope as compared to one application and control plot. They also reported that pesticide application with PB rope also decrease the pink bollworm infestation and it was found lowest in PB-Rope + Spintoram treatment as compared to PB-Rope + Triazophos, and Bifenthrin (Qureshi et al., 2020). Another research study demonstrates that the application of PB rope dispenser (@100/acre) at the pin square stage resulted in lower damage to flowers and bolls than control fields (Hussain et al., 2021). It is advised that PB rope dispensers be made commercially available in Pakistan, and farmers must be encouraged to use them.

Sterile Insect Technique

The sterile insect technique (SIT) is a pest control approach in which sterilized male insects are released to mate with wild females, resulting in no offspring and a diminishing pest population. In California's San Joaquin Valley, an effective SIT program was implemented to eliminate pink bollworms. During the cotton-growing season, sterile pink bollworm adults were released daily to reduce their populations (Staten et al., 1993). Similarly, Pink bollworm sterile moths will be released from a Phoenix-based facility at a rate of 100 moths per acre per day, or 25 million moths per day, from the four-leaf growth stage to defoliation or harvest, primarily on conventional cotton fields in the eradication region, with a reduced rate used on Bt transgenic cotton (Staten et al., 1993). As the process continues or is repeated, the extinction of native insects occurs when the number of sterile insect increases, leading to a decrease in the native population (Knipling, 1979). A native female who mates with a sterile male will lay infertile eggs. This insect control method is (Knipling, 1979). A native female who mates with a sterile male will lay infertile eggs. This insect control method is called the sterile insect technique (SIT).

In Arizona, researchers implemented a multi-tactic eradication program that deploys sterile insect releases rather than refuges to postpone pink bollworm resistance to Bt cotton. This technique for delaying resistance is based on the assumption that sterile males of pink bollworm (released) will mate mostly with rare resistant adults emerging from Bt cotton, producing no fertile offspring (Tabashnik et al., 2010). This computer simulation demonstrates that this strategy works in principle, even when pest resistance is dominantly inherited (Tabashnik et al., 2010). Tabashnik et al., (2021) and his colleagues employed the sterile insect release synergistic with transgenic Bt cotton to reduce the pink bollworm population from over 2 billion to zero between 2005 to 2013 in cotton-growing areas of the USA and northern Mexico. The eradication of this pest saved farmers $192 million from 2014 to 2019. It also facilitated a reduction in insecticides (82%) while also providing eco-friendly management of pink bollworm.

Other field trials show that an engineered strain of the Sterile Insect Technique (SIT) performs well under field conditions, with attributes like mate finding, copulation, dispersal, and persistence comparable between the genetically engineered strain and a standard strain (Simmons et al., 2011). Pink bollworm lethal strains with partial mortality have been developed using RIDL (Release of Insects with Dominant Lethality) technology, a control strategy involving genetically engineered insects with a lethal gene in their genome. These genes only kill young ones, typically larvae or pupae, and cause death in the organism (Morrison et al., 2012).

The Sterile Insect Technique (SIT) is a promising method for controlling pink bollworm, but faces logistical challenges such as high costs, limited access to radiation facilities, and resistance to radiation. It also requires large-scale coordination and community engagement, making scalability a challenge. Alternative technologies like RIDL are under development, and addressing these challenges requires optimizing mass-rearing, improving insect quality, and integrating SIT with other pest control strategies (Tabashnik et al., 2021). The Sterile Insect Technique (SIT) is an ecologically safe pest management technology that eliminates the need for chemical pesticides, decreases environmental impact, and leaves no hazardous residues. SIT protects biodiversity and promotes climate-smart agriculture by targeting certain pests without eliminating beneficial insects. Furthermore, it encourages sustainable farming by lowering crop losses and increasing output while maintaining ecological balance (IAEA, 2024).

Bio-Pesticides

Pink bollworm populations can be controlled with biopesticides such as plant extracts. Plant extracts are used in pest management to provide natural alternatives to synthetic pesticides. They can have an impact in a variety of ways, including toxicity, repellency, and growth control. Plant extracts that repel pests by producing strong odours or tastes deter pests from feeding and laying eggs. Toxic plant extracts contain bioactive chemicals that disrupt enzyme action, the nervous system, or the insect's cuticle, causing death or reduced function. Plant extracts that influence growth disturb hormonal balance or moulting processes, resulting in abnormal growth, sterility, or developmental stoppage (Hikal et al., 2017). Some plant extracts can induce mortality against pests, as well as reduce their feeding and reproduction activity (Mensah et al., 2014).

Some plant as sophora plant has metabolites that inhabit the enzyme activity which can be used against pest (Hyun et al., 2008). Similarly, wheat and barley has properties of inhibiting digestive enzymes in insects (Wang et al., 2023; Chaudhary and Gaur, 2023). They show promising perspective that metabolites of these plants could be used as a potential solution against cotton bollworm infestations. In a research study, it was documented that Wheat and barley have the high inhibitory activity against *H. armigera* (Chamani et al., 2024).

Pesticides made from plants are less efficient than chemical pesticides, but they are safer and less expensive. Tobacco extract, neem extract, and datura extract shown the greatest reduction in pink bollworm populations (Rajput et al., 2017). A study investigated the effectiveness of extracts from tobacco, neem, and datura plants in controlling cotton-pink bollworm. The results showed that tobacco extract directly applied to leaves had the most significant impact. These extracts remained effective for 48 hours but diminished after, indicating the need for frequent application. They were also found to be environmentally friendly towards natural pest predators. The study found that plant extracts do not negatively impact pest natural predators (Sinzogan et al., 2006), suggesting that integrated pest management can effectively suppress pest populations, minimize non-target effects, and preserve ecosystem integrity by combining bio-pesticides with other management strategies (Bharti and Ibrahim, 2010).

Biopesticides are environmentally safer, effective in resistance management, and integrate well with IPM strategies. They are environmentally friendly, reducing pest resistance. However, their effectiveness is influenced by environmental factors and may require more frequent applications due to their lower persistence. Future research efforts in the field of plant extract-based insecticides should focus on evaluating their stability under field conditions.

Chemical Control

When the pink bollworm population exceeds the economic threshold level (ETL) of 10% boll or flower damage in August and 5% boll or flower damage in September and October), the use of insecticides becomes a last option. Conventional pest management relies heavily on chemicals or insecticides to control the pest. In cotton, most farmers spend most on insecticides for bollworm management but all practices are in vain. It is due to pest-hidden feeding behavior i.e. inside the bolls and more adaptability of pink bollworm against insecticides like detoxification etc.

To protect the beneficial fauna, novel pesticides should be used instead of harsh pyrethroids in the outset. Pesticides indicated for pink bollworm are listed as follows: Pesticides of the synthetic pyrethroid Group such as bifenthrin (@330 mL per acre), lambda cyhalothrin (@330 mL per acre), deltamethrin (@330 mL per acre), and gamma cyhalothrin (@125 mL per acre). Pesticides of the Organophosphate group such as triazophos (@800 ml per acre), profenophos (@800 ml per acre), and chlorpyrifos (@800 ml per acre), new chemistry pesticides include spintoram (@100 ml per acre), spinosad (@50 ml per acre), and chlorantraniliprole (@50 ml per acre) and use mixtures of pesticides as 500 ml of Deltamethrin + Triazophos and 500 ml per acre of Cypermethrin + Profenofos can also be used (Arshad, 2025). According to a research, plots treated with thiodicarb, spinosad, and indoxacarb had substantially less locule damage from pink bollworm than the control plot (Shivanna et al., 2012). In a laboratory experiment, Sabry, (2013) found that lambda-cyhalothrin performed better results than thiamethoxam and ibuprofen against freshly hatched pink bollworm larvae. Similarly, Spinosad (45 SC), emamectin benzoate (5 SG), and chlorantraniliprole (20 SC) have been shown to reduce the population of pink bollworm larvae as well as reduce boll infestation (Zaki and Hegab, 2015; Mahalakshmi and Prasad, 2021).

The research study was conducted in India and found that all chemical insecticide treatments were superior over untreated control. Chlorantraniliprole 18.5 SC @ 55 g a.i./ha was most effective against pink bollworm, resulting in the lowest green boll damage, open boll damage, and highest seed cotton yield. The study also found that chlorantraniliprole was safer to natural enemies, with a higher number of enemies per plant (Naik et al., 2023). In another study emamectin benzoate was found to be the most effective in reducing larval population, causing green boll damage, and maximizing seed cotton yield (Kaur and Kaur, 2024). In this study, spinetoram was also found the second most effective insecticide. A field study evaluating insecticides and botanicals against pink bollworm in cotton showed emamectin benzoate 5SG as the most effective treatment. It reduced boll damage by 89.25% and yielded the highest (Shree et al, 2024). Among botanicals, 5% NSKE was the most effective, with a damage reduction of 58.06% over control.

Several variables restrict chemical control's efficiency against pink bollworm. Continuous usage of pesticides can result in resistance development, lowering their long-term efficacy. Chemical treatments can carry environmental dangers, increasing pollutants and hurting vital insects. Synthetic pyrethroids may increase pest populations in cotton crops, leading to imbalances in the agro-ecosystem and resistance issues. Alternatives are needed to minimize harm to humans, livestock, and non-target organisms (Kumar et al. 2019). Emamectin benzoate, a neurotoxic insecticide, is more selective against Lepidoptera and less harmful to beneficial arthropods compared to broad-spectrum compounds. Newer insecticides, such as avermectin, are safer for non-target organisms and natural enemies (Cruces et al., 2021).

Another problem is pink bollworms' hidden feeding behavior inside cotton bolls, which makes it difficult for pesticides to access and remove the larvae successfully. Given the limits of chemical control, applying IPM tactics is critical to effective pink bollworm management. One important strategy is to employ Bt cotton, especially transgenic types such as Bollgard II, which helps lower pink bollworm populations by releasing insecticidal proteins but cultivate it by following proper refuge strategy. Pheromone traps also serve an important function in monitoring insect activity and interrupting mating, which helps to reduce population numbers. Cultural measures, such as adequate field cleaning and timely harvesting, contribute to reducing insect habitats and limiting infestations. Farmers may accomplish long-term and successful pest control by combining these measures, minimizing their dependency on chemical pesticides.

CONCLUSION

It was concluded that integrated pest management (IPM) for cotton *P. gossypiella* provides a multidimensional strategy for sustainable pest control. By use of cultural control measures such as early planting, and trapping crops can interrupt *P. gossypiella* lifecycles. Farmers can also lower *P. gossypiella* populations while preserving environmental balance by integrating natural enemies and using sex pheromones or PB ropes appropriately. Using proper refuge methods is a viable method for managing *P. gossypiella* infestation in transgenic cotton with promising sustainable pest management practices. IPM has economic benefits such as reducing reliance on pesticides, increasing yields, boosting farmer livelihoods, and minimizing environmental impacts. Sustainable IPM requires continuous monitoring, prompt decisions, and farmer education. Collaboration among stakeholders is necessary for maximizing IPM's potential in combating pink bollworm and ensuring the future of the cotton industry.

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AUTHOR CONTRIBUTIONS

All authors have contributed equally.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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