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Sustainable pest management techniques for the control of pink bollworm, *Pectinophora gossypiella* (Saunders) in cotton

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Abstract

The pink bollworm, *Pectinophora gossypiella* is the essential pest in cotton production areas i.e. southwestern United States and many other cotton-producing areas of the world. The high costs of chemical control, ongoing economic losses, secondary pest problems and environmental deliberation propose the need for ecologically oriented Pink Bollworm management strategies. Extensive research has occurred in a wide array of monitoring, biological control, cultural, behavioural, and genetic and host plant resistance methods which can serve as a base for the application of integrated Pink Bollworm management systems. Pink Bollworm life history characteristics, as the high of adults is specific, indicates the need for combinations of selected integrated pest management components implemented over large geographical area. Such areas associated a vast range of Pink Bollworm population densities, differences in cotton production methods and social and environmental considerations. The foremost option is tailor-made systems for targeted management areas with the selection of IPM components formed on the PBW population density, crop production methods and economic viability. The unlikelihood of eradication specifies the need for long-term monitoring and programme maintenance following successful area-wide management. PBW success of area-wide management is highly dependent on participation in the planning, site selection, implementation and evaluation phases of the programme by all segments of the agricultural community. An essential component deals with highly effective extension education communication programme. A local uncoordinated effort does not decrease the economic status of this pest in any area where it is an established pest. The possible long-term benefits of PBW population suppression on an area broad basis emerge to justify area broad efforts in terms of decreased costs, more effective control, less environmental contamination and other outermost problems related with traditional control approaches. Transgenic crops that produce *Bacillus thuringiensis* toxins kill some important insect pests and thus can decrease confidence on insecticides. Extensive planting of such Bt crops increased concerns that their convenience would be cut short by quick evolution of resistance to Bt toxins by pests. Since 1997, *Pectinophora gossypiella* is a almost pest that has experienced enormous selection for resistance to Bt cotton in Arizona.

Keywords: Pink bollworm, IPM module, transgenic, *Bacillus thuringiensis*

1. Introduction

Cotton (*Gossypium spp.*) is the most leading avaricious fiber crop of India, producing natural fiber, fuel and edible oil, plays an important role in Indian economy (Prasad *et al.*, 2018) [70]. On an area of 12.43 mha with average productivity of 505.46 kg/ha in India it is cultivated (Anonymous, 2018) [4]. In terms of land India is the largest cotton cultivating country and second largest in terms of production in the world. India accounts for 34 % of the cotton area and 20 % of the cotton production in the world. In terms of productivity, but India ranks only 46th with a yield of about one tonne /hectare (FAO, 2011) [24]. The yield of cotton is one of the lowest among the foremost cotton producing countries in the world. China, USA and Pakistan are the other utmost cotton producing countries in the world. In India there are about nine huge cotton growing states with more than one lakh hectare area under cotton two states, Maharashtra and Gujarat, alone account for nearly 58 % of the cotton area in the country. Gujarat alone accounts for nearly 32 % in terms of production, in the country though it has only 25 % of the cotton area. It is because of excessive productivity of cotton in Gujarat. In terms of productivity Gujarat ranks 3rd in the country after Punjab and Haryana... On the other hand, Maharashtra with 33 % of the area accounts only for 21 % of the cotton production because of low productivity. The productivity of Maharashtra is one of the lowest in the country. Tamil Nadu has a relatively lower share in production but ranks fourth in terms of productivity in India. In India Cotton is mostly grown as a rainfed crop.

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The cotton crop around 64 % in the country is grown without irrigation. The range of irrigation differs broadly across states, with Punjab growing the total cotton crop under irrigation and Maharashtra growing nearly 95 % of the crop under rainfed condition. At the all India level only 36 % of the cotton crop is irrigated been most evident for genetically modified crops. This is the quickest diffusion of any latest crop technology in the history of mankind (Qaim, 2005) ^[71]. The number of countries growing biotech crops has expanded regularly from 6 in 1996, the first year of commercialisation, to 18 in 2003 and 29 in 2010. The growth rate between 1996 and 2010, the 15th anniversary of the commercialization of biotech crops, was an unmatched 87 double improve thus making it the fastest adopted crop technology in recent history. In India 6.3 million small farmers exploited from planting 9.4 million hectares of Bt cotton, correspondent to a high adoption rate of 86 % (Clive, 2010) ^[15]. Various biotic and abiotic stresses are restraints in cotton production including the bollworm complex and sucking pests (Kranthi; *et al.*, 2002). Earlier, in non Bt cotton, the infestation of pink bollworm caused 2.8 to 61.9 % vloss in seed cotton yield, 2.1 to 47.10 % loss in oil content and 10.70 to 59.20 % loss in usual opening of bolls (Patil, 2003) ^[67].

Pink bollworm, *P. gossypiella* is the leading lepidopteron pest of the cotton growing area and cause significant loss around the season. The pest originates from South-Asian part of the world and was first described from India in 1842 from cotton and by mid-nineties subsequently spread across leading cotton growing parts of the world (Byers and Naranjo, 2014) ^[10]. However, Indo-Pakistan origin of this pest was reconfirmed recently (Sridhar *et al.*, 2017) ^[85]. Female moth lay eggs on squares, flowers or green bolls. The destructive larvae of pink bollworm usually feeds on flower buds, bolls and seeds therein, which results in malformation, rotting, premature or partial boll opening, reduction in fibre length and throughout deterioration in the quality of cotton crop due to staining of the lint. The larval stage is normally unseen within the cotton fruiting bodies making them unavailable by insecticidal sprays due to which its management is a hard task for cotton growers. This indicates the significance of this pest in cotton production system. Pink bollworm endure to be the pest of global concern with its most destructive nature of feeding habit known to cause economic loss in seed cotton yield to the extent of 2.8 to 61.9 %, reduction in oil content to the tune of 2.1 to 47.1 % and 10.7 to 59.2 % inferior opening of bolls (Shrinivas *et al.*, 2019) ^[83]. The occurrence of pink bollworm in many hotspots created serious boll damage and some extent yield losses were also discovered in Bt cotton and improved in management costs due to pink bollworm in parts of Gujarat, Andhra Pradesh, Telangana, Maharashtra and Karnataka (China Babu *et al.*, 2017) ^[13]. The fast escalation of the issue mainly attributed to the failure of refuge, too many Bt hybrids of different durations, mono-cropping with addition of crop season, absence of proper monitoring of bollworm and its resistance to gene, near monophagy character of pink bollworm and compatible climate (Desai *et al.*, 2015 and Kranthi, 2015) ^[18]. After initial introduction of Bt-cotton as Bollgard I in 2002 and eventually Bollgard II in 2006, the cotton crop could resist the bollworms upto 2010. The first occurrence of pink bollworm on Bollgard-II was reported from Amreli in Gujarat which revealed mean survival of 72% at diagnostic concentration Cry1Ac (Dhurua and Gujar, 2011) ^[21]. Successive research studies from Monsanto in 2010 proved the Cry1Ac resistance reporting unfamiliar survival of pink bollworm on Bt cotton during 2009 in four districts of Gujarat i.e. Amreli, Bhavnagar,

Junagarh and Rajkot. Diet incorporation bioassays to assess resistance levels of -1 Cry1Ac at diagnostic concentration (10 µg ml) in two populations collected from Bt cotton (Anand, Gujarat) and non-Bt cotton (Akola, Maharashtra) fields during 2010-11 were done. The population collected on Bt cotton revealed survival of 65% whereas complete mortality was noticed in non-Bt field collected population (Fabrick *et al.*, 2014) ^[24]. Recently, pink bollworm resistance in Bt cotton to both Cry1Ac and Cry2Ab has been reported from India suggesting future threat to cotton cultivation (Naik *et al.*, 2018) ^[61]. To address this dual problem of resistance to Bt toxins and ineffectiveness of insecticides to extend target insect due to concealed feeding habit of pink bollworm (Lykouressis *et al.*, 2005) ^[51], there is a need to develop an substitute control procedure for its management.

2. Biology of pink bollworm on artificial diet under controlled condition

Pink bollworm is the leading lepidopteran pest of the cotton growing regions and cause significant infestation all over the season. The occurrence of pink bollworm in many hotspots created serious boll damage and some extent yield losses were also discovered in Bt cotton and increased in management costs due to pink bollworm in parts of Gujarat, Andhra Pradesh, Telangana, Maharashtra and Karnataka (China Babu *et al.*, 2017) ^[13]. The fast intensification of the issue mainly attributed to the failure of refuge, too many Bt hybrids of different durations, mono-cropping with addition of crop season, absence of proper monitoring of bollworm and its resistance to gene, near monophagy nature of pink bollworm and compatible climate (Desai *et al.*, 2015 and Kranthi, 2015). (Muralimohan *et al.*, 2009) ^[18] had developed two-phase diet for rearing neonate and other instars of pink bollworm while studying bioassay of pink bollworm in case of cry proteins. They systematized the two phase diet for mass rearing of larvae of pink bollworm consist of first 10 days rearing of larvae on diet of cotton seed and chickpea flour and successive rearing on okra fruits. They found shortest larval (21.34±2.61days) and pupal period (7.96±1.37 days) as well as maximum adult emergence (91.66%) with identical sex ratio on this two phase diet. At Sindh in Pakistan, (Shah *et al.*, 2013) studied the biology of pink bollworm at three different temperatures (27, 31, and 35±1°C) on their natural diet cotton bolls, flowers and squares and found highest development rate at 27±1 °C while lowest development rate at 35±1°C. They also noticed 69.5 % cumulative survival and highest mortality in 1st larval instars and lowest mortality in 4th larval instars. (Dharajothi *et al.*, 2016) ^[20] standardized the cost effective and easily available ingredients based artificial diet for continuous rearing of pink bollworm at CICR, Coimbatore, India and they found 95.56 % larval recovery, per cent pupae formation, adult emergence and egg hatchability. The eggs, larval and pupal periods were set down to be 4.8±0.632, 25.10±0.994 and 7.9±0.88 days, respectively. Larval and pupal weights were set down as 21.40±3.63, 18.00± 2.73 mg, respectively. Recently, the biology of pink bollworm have been carried out in the laboratory under controlled condition temperature 28.34±3.15 °C and relative humidity 40.00±7.20 % on their artificial diet.

3. The halo effect: suppression of pink bollworm cotton by Bt cotton

Transgenic corn and cotton give rise to insecticidal proteins from *Bacillus thuringiensis* (Bt) were planted on more than 66 million hectares worldwide top control insect pests and decrease reliance on insecticide sprays (James and Sanahuja,

2011) [78]. Field-evolved resistance creating reduced efficacy of Bt crops has been documented for some populations of many major target pests (Kruger 2009; Tabashnik 2008 and 2009; Storer, 2010; Dhurua, 2011, Gassmann, 2011 and Tabashnik, 2010) [47, 21, 90, 91, 92, 87, 28]. The foremost approach for delaying pest resistance to Bt crops is planting refuges of non-Bt host plants near Bt crops to encourage survival of susceptible pests (Tabashnik, 2008 and 2009) [90, 91]. One of the potential drawbacks of the refuge strategy is increased pest damage to non-Bt crop plants in refuges (Tabashnik, *et al.* 2010) [91]. This “halo effect” was predicted on theoretical grounds, because females emerging from non-Bt crops lay some of their eggs on nearby Bt crops, and the larvae hatching from such eggs suffer high mortality on the Bt crops (Tabashnik, 2010; Carriere, 2003) [91, 90]. If Bt plants account for a substantial percentage of the available host plants, regional pest populations can be greatly reduced, resulting in less damage to non-Bt plants (Tabashnik, 2010) [91]. By reducing damage to non-Bt plants, the halo effect can reduce the need for insecticide sprays on non-Bt crops and encourage compliance with the refuge strategy, thereby increasing the benefits and sustainability of Bt crops (Tabashnik, 2010; Hutchison, 2010) [91]. The halo effect was first registered for pink bollworm, *Pectinophora gossypiella* (Carriere, 2003) [11], a worldwide pest that has several probable host plants, but feeds almost exclusively on cotton in the United States and China (Wu, 2008) [104]. In the United States, planting of non-Bt cotton refuges was the primary strategy for delaying pink bollworm resistance to Bt cotton (Tabashnik, 2010) [91]. The outcome from modeling suggested that regional suppression of pink bollworm in non-Bt cotton would take place when the percentage of cotton planted to Bt cotton exceeded a threshold value. Investigation of 10 years of field data encompassing five years before and after adoption of transgenic cotton producing Bt toxin Cry1Ac in the state of Arizona in the southwestern United States supported this idea. In particular, the Arizona field data suggested that regional suppression of pink bollworm occurred when the percentage of cotton planted to Bt cotton exceeded a threshold of approximately 65% (Carriere, 2003) [11]. Here we tested the hypothesis that Bt cotton suppressed pink bollworm populations on non-Bt cotton in the Yangtze River Valley, a utmost cotton growing-region of China (Wu, 2005) [104]. We analyzed pink bollworm population density in six provinces of the Yangtze River Valley during 16 years, including five years before Bt cotton was adopted. In these six provinces, the percentage of cotton hectares planted with Bt cotton increased from 9% in 2000, to 62% in 2005, 84% in 2006, and 94% in 2009 and 2010. We found that the population density of pink bollworm on non-Bt cotton was 91 to 95% lower in 2010, after 11 years of Bt cotton, compared with the mean population density during the five years before Bt cotton. Compatible with results from Arizona, the yearly per capita growth rate (r) was lower when the percentage of cotton planted to Bt cotton overshoot 65%. In addition, in 2010 compared with the eight years before Bt cotton adoption, insecticide sprays targeting bollworms on cotton reduced by 69%.

4. Evidence for population expansion of cotton pink bollworm Pink bollworm has become evident as a threat to cotton cultivation in south and central cotton growing zones of India where the pest has evolved resistance to Cry 1Ab expressing cotton also developing resistance to insecticides and infesting late season cotton (Naik, 2018; Kranthi, 2012) [61]. The Pink

Bollworm presumes as major pest status even in some regions of northern India where there are ginning and oil extraction units which are acquiring cotton seeds from central and south Indian cotton states where PBW has demonstrated resistance to Cry toxins in the field. The evolution of resistance and pest conversion to Bt crops containing Cry1Ac and Cry2Ab has been discovered recently (Tabashnik, 2015; Tay, 2015 and Tabashnik, 2017) [93, 32]. The development of resistance is due to multiple factors such as absence of refuge (Wan, 2012) or supply of fraudulent refuge13, mono cropping, cultivation of long duration hybrids, extended cropping season (Kranthi, 2017). Bollgard-II (BGII) cotton conveying two proteins, Cry1Ac and Cry2Ab invades approximately more than 90% of the area cultivating *G. hirsutum* cotton in India. BGII was awaited to be effective against the pink bollworm especially after resistance to the single gene Cry1Ac was turned up as heavy field infestations of PBW in Bollgard (BG), that was confined to Gujarat state (Mohan, 2016) [56]. Despite reports of reasonable breakdown of BGII resistance the contribution of stakeholders of the technology was unacceptably insufficient to certify its sustainability (Fabrick *et al.*, 2015) [23]. Pink bollworm (PBW) adaptation to transgenic Bt-cotton expressing Cry1Ac (BG) and ‘Cry1Ac+Cry2Ab’ (BGII) was evaluated in 10 major cotton-cultivating states of India. However the PBW larval incidence during this period on Bt-cotton was found less in north cotton cultivating zone of India, where as in central and south India, PBW larval recovery from BGII cotton bolls was excessive in the range of 28.85-72.49% (Naik, 2018) [61]. PBW infestation causes cavity damage of 37.5% and 13.58% on non-Bt and Bt cotton respectively, at about 160 days of planting (Naik *et al.*, 2014) [61]. Presently PBW is assuming a major pest status even in northern India where it had minor pest status earlier. Recently the pink bollworm strain having 300-fold resistance to Cry1Ac, 2.6-fold cross-resistance to Cry2Ab identified and analyzed with novel cadherin allele (r16) builds its life cycle on transgenic Bt cotton containing Cry1Ac (Wang *et al.*, 2019) [109]. Mitochondrial DNA (COI gene) is maternally inherited, well conserved and evolves in a nearly neutral fashion so it reflects the divergence times, which makes it a robust marker for determining genetic relationships and geographical studies (Birmingham, 1993; Avise, 2000; Armstrong, 2005; Galtier, 2009 and Prabhakar, 2013). Further the genetic constitution of a pest population is very vital in determining its capacity to tolerate adverse climatic conditions and adoption to new conditions (Hayden, 2011). Population genetic structure and genetic diversity defines the level of adaptation of a population to environmental change and susceptibility to selection pressure (Pauls, 2013). Gene flow through dispersion and migration which is responsible for determining genetic variation leads to evolution of local populations (Kremer *et al.*, 2012). Even in some of the lepidopteran species, the genetic diversity and genetic structure are reported to be related to their migration capacity as well as number of generations (Chen, 2010 and Men, 2013). Studies on population structure and genetic diversity of PBW has been explored more in Asiatic countries such as India, Pakistan and China owing to the development of resistance to Bt cotton by PBW populations (Liu, 2010; Wang *et al.*, 2010 and Sridhar *et al.*, 2017) [85]. (Liu *et al.*, 2009) studied the population genetic structure of Chinese PBW using mitochondrial COII and Nad4 primers, and found extremely low genetic variability among all populations examined from nine provinces of China. Sequence variation

in the Nad4 region differentiated the Chinese populations from the Pakistani and American populations. Haplotypes and differentiation in PBW populations of China was identified using piggyBac-like elements (Wang *et al.*, 2010) [101]. (Sridhar *et al.*, 2017) [85] based on the analysis of pink bollworm population, and based on haplotype diversity results opined that the populations might be experiencing population expansion but could not provide the evidence through neutrality tests owing to the small population size.

5. Seasonal incidence of Pink bollworm on Bt Cotton

Among the array of insects, especially the bollworms (Dhurua & Gujar 2011) [21] viz., American bollworm, *Helicoverpa armigera* (Hubner), Spiny bollworm, *Earias insulana* (Boiusduval), Spotted bollworm and Pink bollworm normally cited as bollworm complex, pose greater threat to cotton production (Ghosh, 2001; Kranthi, 2015) [29]. The management of insect pests through synthetic insecticides was accomplished and considered as a privilege during the green revolution era. The one and only reliance on synthetic insecticides particularly pyrethroids (Ramasubra-manyam, 2004) [73] caused an imbalance in the agro-system creating resistance and revival problems undertaking substitute control measures (Prasad, 2018) [70]. On average, farmers apply 6 to 8 rounds of insecticides in the rainfed situation and 12 to 18 rounds in the irrigated situation (Kulkarni *et al.*, 2003). Out of this, bollworm control alone takes about 80 % of the insecticides worth of around 12 billion rupees and accounting for about one-third of current pesticide sales (Gupta, 2001). To reduce pesticide usage on cotton, as an alternative approach to manage bollworm, the thrusting of a foreign gene through genetic engineering and evolving transgenic cotton regarded as an important milestone for the management of major pests particularly bollworms without injurious effects on ecosystem (ISAAA, 2016). Transgenic cotton containing single toxin (Cry 1Ac) Bt-1 has been commercialized during 2002 to afford protection against cotton bollworms. Further, to increase the efficacy and durability of the GM technology for bollworm control, second-generation GM-Bt cotton (Bt-II) conveying two Bt proteins, Cry1Ac + Cry2Ab, was introduced into India (Choudhary, 2013) [14]. PBW populations were reported to have developed resistance to Cry1Ac and were found to survive on Bt-I cotton fields in Gujarat State in India, but were being successfully controlled by the dual-gene Bt-II cotton (Dhurua & Gujar 2011) [21]. However, Surveys conducted across India showed progressive increases in the survival rate of PBW larvae in green bolls of Bt-II cotton F1 hybrid varieties (Vakudavath, 2018) [99]. In Bt cotton, the expression of cry protein toxin varied all over the cropping period and declines after 85-100 DAS in the plant system due to abiotic factors (Kranthi *et al.*, 2002) distinctly under moisture stress and poor soil nutritional condition (Blaise *et al.*, 2011) [9].

Percent Rosette flower: The observations on rosette flower due to pink bollworm infestation were recorded from 60 DAS in fortnightly intervals on 50 randomly selected plants. Later the total numbers of flowers and rosette flowers were counted and the percent rosette flowering was work out using the given formula

Rosette flower (%) = Total no. of rosette flowers per plant / Total no. of flowers per plant × 100

Larval population in green bolls: The observations on the occurrence of PBW in intervals. For this purpose, 50 tender green cotton bolls of three-week age was collected and brought to the laboratory for further observations. Each tender green boll was cut opened along with ridges of the locules with the help of sharp cutter to see the presence of larvae. Finally, the total number of pink bollworm larvae per 50 green bolls was worked out using the following formula.

Larval population (%) = No. of larvae in green bolls / Total no. of green bolls × 100

6. Compatibility of entomopathogenic fungi and *Azadirachta indica* extract against the cotton pink bollworm, under controlled conditions

Different approaches such as chemical insecticides and growing resistant cultivar (transgenic Bt cotton containing Cry1Ac toxin) have been used to manage the pest control (Heuberger *et al.* 2014) [32], but they have not given superior control levels of the pest (Mohamed *et al.* 2016) [55]. Plant extracts such as *Nicotiana tabacium* and *Azadirachta indica* have broadly been used to control insect pests. *A. indica* has been used for years in Indo-Pak against many insect pests and is still used for stored grain pest (Rajendran and Sriranjini, 2008). Due to its broad host range, low cost production and unarmful impact on environment (Mathew 2016) [52] makes it a safer substitute method to control some insect pests. The entomopathogenic fungi are between the most effective and environmental friendly biological control agents that invade their host insect through the cuticle and play a key role in the regulation of insect pest population in natural ecosystem (Niu *et al.*, 2019). EPFs can be used against a wide range of insect pests and their nonspecific actions and antagonistic natures give them broad host range ability (Ong and Vandermeer 2014). More than 700 species of fungi belonging to 90 genera among *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, *Purpureocillium lilacinum*, and *Isaria fumosorosea* are the widely used ones as biological control agent against many agricultural pests (Rizwan *et al.* 2019) [75]. The expansion of plant removes which go about as both adjuvant (Nursal and Ilyas 2019) [63] and bio-pesticide (Dougoud *et al.* 2019) [22] can upraise the coverage of leaf and persistence of EPFs (Swiergiel *et al.* 2016) [88] resulting in enhanced performance of EPFs and plant extracts in combination for the suppression of some insect pests such as *P. gossypiella* (Vashisth *et al.* 2019).

6.1 Entomopathogenic fungi: Two commercial formulations of 3 EPFs viz. *V. lecanii*, *M. anisopliae* and *B. bassiana* were acquired from AgriLife SOM Phytopharma (India) Limited® (www.agrilife.in) in the form of talc powder. Formulations, at 2 different concentrations (1 × 10⁶ and 1 × 10⁸ CFU/ml), were tested against *P. gossypiella*. Hemocytometer and potato dextrose agar (PDA) were used to determine the conidial concentrations and germination in conidial suspension, respectively. Measurement of conidial germination was figured by randomly counting 200 conidia in each plate at 25 ± 2 °C, 18 h after incubation (Atta *et al.*, 2020) [6].

6.2 Preparation of *Azadirachta indica* plant extract: Plant extract of *A. indica* was prepared by embracing the methodology of (Ali *et al.*, 2017). Fresh collected leaves of *A. indica* were sufficiently washed by distilled water and dried in

shadow, followed by electric grinding to get fine powder. A Fine *A. indica* powder (50 g) was dissolved in distilled water (500 ml) in a 2.5 liter sized conical flask by heating the solution at 60 °C and shaking the flask continuously with a magnetic stirrer for 6 h. Solution was filtered, using Whatman no. 1 after sieving with muslin cloth to remove any solid particles. Rotary evaporator was used to evaporate the solution in vacuumed conditions in hot air oven at to bring the dry plant extract to a constant volume 50 ml. The solution thus obtained was considered as 100% *A. indica* extract, stored at 4 °C for further investigations.

7. Efficacy of botanicals and biopesticides on population dynamics of bollworm complex and their safety to the predators in non Bt cotton

Amidst the bollworms as complicated, American bollworm, spotted bollworm and pink bollworm are of uniform occurrence on non-Bt cotton. Chemical insecticides were examined as the only alternative for the management of bollworms on non-Bt cotton. However, it has been observed that sufficient control of bollworms could not be accomplish due to resistance to several insecticides. Biorational and microbial pesticides have been supported as suitable alternatives, because of biosafety and environmental safety. Current investigation was conveyed out to evaluate the performance of different botanicals and biopesticides for the management of complex network of bollworms in cotton. A research trial on management of bollworm complex of cotton with plant products was conveyed out a view to assess the performance of herbal extracts such as HaNP, *Bacillus thuringiensis* and spinosad on cotton bollworm complex and their natural enemies. Twenty different treatments consisting of NSE 5%, neem oil 1%, synthetic neem formulation (Azadirachtin 1500 ppm) @ 2ml/lit, CASE 5% and untreated control were evaluated initially for sucking pests and continued for bollworm complex management with biopesticides. The treatments undertaken for sucking pests continued for bollworms followed by HaNPV 250 LE/ha, Bt 1000 g/ha and spinosad 45 SC @ 0.01% (0.2 ml/lit) along with untreated control. The treatment sprays for bollworm management were undertaken from the initiation of the damage and were repeated at an interval of 10 days. Four sprays were for sucking pests and three sprays for bollworm complex were given on a plot size of 6.0 m x 4.8 m (28.80 sq m). Following observations were undertaken to study population dynamics. Observations on the number of eggs, larvae of *H. armigera* per plant were recorded from randomly selected five plants from each net plot at 3, 5 and 10 days after spraying and the average egg population per plant was worked out. Incidence of *P. gossypiella* was taken down by removing 15 green bolls from the border line plants at 105, 120 and 135 days old crop. These bolls were examined out and noticed for the presence of *P. gossypiella* and per cent infestation worked out. Observations were made on the population of eggs and larvae of *Chrysoperla zastrowi sillemi*, larval and adults of *Cheilomenes sexmaculata* and spiders on randomly selected five plants from each whole plant at 3, 5 and 10 days after each spray during both the years. These observations were analyzed for each year and also the two years data were pooled for analysis using ANOVA. The pooled data on egg population revealed that marginal effects in reducing the *H. armigera* egg population over control plots was found in all treatments. It was also been noticed that the egg population was kept at minimum up to 3 DAS (days after spray), which increased slightly at 5 and 10 DAS. The application of NSKE 5% and azadirachtin 1500

ppm followed by HaNPV proved slightly better by recording 0.39 and 0.41 egg per plant at 3 DAS, respectively and found equal with NSKE 5% followed by spinosad (0.40 egg/plant). The egg population observed in HaNPV treatment was comparable with the findings of (Ameta *et al.*, 2004) [2]. The botanicals, NSE 5% and azadirachtin 1500 ppm had the identical effects in recording the egg population between 0.44 and 0.46/plant, 3 DAS and is comparable with findings of (Panickar *et al.*, 2003) [65] who reported ovicidal effect on *H. armigera* eggs with commercial azadirachtin. The data on larval population of *H. armigera* depicted that most of the treatments have shown similar effect as in case of egg count. The lowest larval population was observed in NSE 5% and Azadirachtin 1500 ppm followed by spinosad (0.41 and 0.42 larva/plant) on 5th DAS and was statistically similar with NSE 5% and Azadirachtin 1500 ppm followed by HaNPV (each 0.47 larvae/plant). The sole application of spinosad as well as sole application of NSKE 5% have shown similar performance in containing the larval population on 5th DAS observation on 10th DAS revealed that the application of botanicals followed by biopesticides have proved better over the sole application of botanicals. Minimum larval population with Azadirachtin and spinosad was reported by (Dandale *et al.*, 2004) [17] and (Patil *et al.*, 2004) [66]. Likewise, the lowest population of *H. armigera* by the application of spinosad was reported on cotton crop with NSKE 5% by (Sarode *et al.*, 1995) [79]. The treatments showed maximum effectiveness on 5th day as compared to 3rd and the population of larval of *E. Vitella* increased on 10th day of observation. The lowest larval population was observed with NSE 5% and Azadirachtin 1500 ppm followed by spinosad recording 0.32 and 0.34 larva/plant on 5th DAS, which were on par with NSE 5% and Azadirachtin 1500 ppm followed by Bt (0.37 larva/plant for each treatment). The sole application of NSE 5% as well as spinosad on untreated control have shown reduction in the larval population of *E. vitella* up to 0.40 and 0.54 larva/plant on 5th DAS. The applications of botanicals followed by biopesticides have proved better over the sole applications of botanicals. (Dandale *et al.*, 2004) [17] reported effective results with Azadirachtin and spinosad as well as Azadirachtin and Bt and (Jeyakumar and Gupta, 2002) [37] found superior results with the application of Azadirachtin and Bt. The application of NSE 5% and azadirachtin 1500 ppm followed by spinosad emerged as the best treatments by recording the lowest larval population of *P. gossypiella* of 0.15 larvae for each. Treatments with neem oil 1% and CASE 5% followed by spinosad recorded 0.18 larvae per green boll for each treatment and these treatments were found statistically similar. The sole applications of spinosad and Bt also recorded less population of pink bollworm larvae (0.21 larva /green boll) and were found on par. The effectiveness of spinosad against pink bollworm have been reported by (Gopaldaswamy *et al.*, 2000) [30] and (Ulaganathan and Gupta, 2004) [98] who observed the minimum population in module consisting of neem products and spinosad as well as Bt. Whereas, (Jeyakumar and Gupta., 2002) [37] found the minimum larval population in Azadirachtin and Bt.

8. Varietal selection, cultural practices and new agronomics systems

The role of classical varietal selection needs to be acknowledged alongside that of transgenic plants and of cultural practices. By the end of the 19th century the growing of short season cottons was recommended in Texas to limit the effects of the boll weevil (King *et al.*, 1996) [77]. There are many examples of the selection of disease resistance against bacterial or cryptogamic diseases (Hillocks, 2000) [33], of

which the widespread use across Africa of bacterial blight tolerant 'Albar', varieties developed in Sudan is one of the best known. The principal characters selected for insect resistance are the gossypol gland density, nectariless, okra leaf shape, frego bract and leaf hair and their combinations (Scheffler *et al.*, 2004) [80]. Today the focus is on the development of cultivars which are adapted to specific growing systems, thanks to the on-going research into the interactions of genotype x growing system (Belot *et al.*, 2005; Constable, 2000) [7, 16]. Earliness remains a principal research preoccupation. Maximising the benefits of earliness requires the judicious management of agronomy, sowing dates, irrigation practices, fertilization, and the use of chemical growth regulators. Plant architecture is another consideration, with interest in narrow-row or ultranarrow-row cotton cultivation practices, especially in the Xinjiang Northwest inland cotton region of China where around 1 million hectares of cotton is grown this way using plastic film mulching to improve emergence rates and weed control. These systems are now finding favour in Argentina, Australia, Brazil, the USA and other countries, thanks to the opportunities provided by the application of herbicide on GM herbicide tolerant varieties (Rossi *et al.*, 2004) [76]. Use of this technique shortens the growing season by 2–3 weeks, while providing superior yields; always assuming that an appropriate management system is in place, frequently with the use of growth regulators and stripper-shaker harvesters. The phytosanitary consequences of these techniques are as yet poorly understood, but the increase in total root volumes caused by the increased plant density may favour subterranean pests such as nematodes and cryptogamic diseases. This cultural technique is often found in association with low-tillage systems, resulting in a very highly modified physico-chemical environment for cotton growth. In addition to improving the

structure and porosity of soils there is an increase in the diversity and abundance of living organisms in the fields, both of vertebrates and invertebrates (Fawcett and Towery, 2002) [25]. Following studies undertaken in various parts of the US cotton belt, pest populations do not seem to be especially favoured by these practices, with the exception of various species of cutworms, grasshoppers, the three-cornered alfalfa hopper and aphids (McCutcheon, 2000) [53]. There is as yet no definitive set of phytosanitary recommendations to accompany these cultural practices (Stewart, 2003) [86]. However, systematic studies have been undertaken to establish the types of cover-crops favouring the beneficial actions of natural enemies (Tillman *et al.*, 2004) [97]. Direct seeding plays a preponderant role, in various systems depending on the local socio-economic conditions. In the humid tropical climate of the Cerrados in Brazil, a recent spectacular development has involved appropriate rotations, direct seeding under cover crops and careful varietal selection. Two crops are grown successively, soya bean and rain-fed rice as the main crops and maize, sorghum and millet as secondary crops, locally called "safrinhas". Cotton is introduced to the system as a secondary crop, sometimes after the two principal crops, sometimes after the cover crops have produced abundant biomass (Seguy *et al.*, 2004) [81]. Studies are being undertaken to evaluate the phytosanitary implications of the use of the cover crops, which may favour the development of certain pests such as Spodoptera frugiperda (Ratnadass *et al.*, 2006; Silvie *et al.*, 2005) [74, 7]. In Australia, by contrast, it is the desire to find a sustainable solution to phytosanitary problems which has principally guided the development of new agronomic techniques (Tab. 1). These rest mainly on the management of pests through the management of habitats (Deutscher *et al.*, 2005) [19].

Table 1: Putting integrated pest management into practice: major activities for each phase of the cotton crop cycle and 'off-season' (Deutscher *et al.*, modified, 2005) [19]

Phases Objectives	Post harvest	Pre-planting	Planting to 1 flower per meter	1 flower per metre to 1 open boll per metre	1 open boll per metre to harvest
1. Growing a healthy plant	Rotation crop, fertilizer requirements, potential disease risks	Seed bed preparation, cotton variety selection, irrigation management strategy	Planting window, planned treatments, water management	Monitor for crop management, nutrient status, growth control, pest control	Final irrigation decisions, defoliation management, pest management
2. Keeping track of insects and damage	Sample cotton stubble for <i>Helicoverpa armigera</i> p-pupae	Risk of different pests and pest management in pre-planting	Sample for pests and beneficial in cotton and in trap crops	Sample for pests and beneficial and use thresholds and predator/beneficial ratio	Stop treatments at 30-40% bolls open
3. Beneficial insects use them don't abuse them	Plant Lucerne in autumn, discuss an IPM or AWM group	Planting diversified habitats, especially sorghum if <i>Trichogramma</i> releases are planned	If chemical control is required, refer to the beneficial impact table	Consider <i>Trichogramma</i> releases into sorghum, food sprays for beneficial, lucerne management	Encourage beneficial to reduce late season resistant pests
4. Prevent the development of resistance	Pupae bust to control <i>Helicoverpa armigera</i> and mites, plant spring trap crop, attend annual resistance management meeting	Consider Bollgard IIR refuge options, choice of insecticides	Use pest and damage thresholds, follow the IRMs strategy for region for Bollgard II R management	Use pest and damage thresholds, follow the IRMs strategy for region for Bollgard II R resistance management	Use pest and damage thresholds, follow the IRMs strategy for region for Bollgard II R resistance management
5. Manage crop and weed hosts	Weeds and cotton re growth management	Carefully consider summer trap rotation crops	Keep farm weed free	Keep farm weed free	Consider winter rotation crops, keep farm weed free
6. use trap crops effectively	Plant spring trap crop, consider flowering date to time planting	Consider summer trap crop	Consider last generation trap crop	Monitor <i>Helicoverpa</i> populations in summer trap crop	Use biological and cultural methods to destroy <i>Helicoverpa</i> stages
7. Support IPM through communication and training	Consider becoming involved in an IPM or AWM group, consider doing the IPM short course	Communicate to discuss spray management plans, attend training courses	Meet regularly with consultant top discuss IPM strategies and attend local field days	Meet regularly with consultant top discuss IPM strategies and attend local field days	Meet regularly with consultant top discuss IPM strategies and attend local field days

9. Conclusion

The principal industrial crop, often the sole cash source for countless small growers in developing countries, source of economic conflicts in the research into 'fair trade', cotton is also the subject of serious phytosanitary and environmental concerns. These are allied to the importance of yield and quality losses occasioned by the particularly rich, polyphagous pest complex. However, the usage of synthetic insecticides in insufficiently understood production systems led to their abuse. The development of the problem of evolved resistance resulted in a stream of new insecticide active ingredients, which in time resulted in an economic impasse for growers. For crop protection specialists, cotton has for long been considered as a bad example of their discipline. At the end of the 1960's the situation was effectively critical. The intensity of public and scientific opinion against the continued use of intensive chemical pest control was increasing rapidly. In the absence of a comprehensive understanding of the factors influencing the dynamics of pest populations, this led, as in other major cropping systems, to the development of the compromise solution of 'integrated control', intended to exploit natural control systems to the maximum extent possible, supported where necessary by the judicious deployment of chemical insecticides. This proved illusory. In the best cases, it was a form of directed control which prevailed, characterized by risk evaluations on the basis of economic intervention thresholds, which were then used to justify each chemical application. The adoption of such measures is indicative of the real difficulties in the practical application of more knowledge-intensive integrated pest management systems. Focusing from the outset on the use of intervention, thresholds has had the perverse effect of re-enforcing the habitual recourse of growers to synthetic pesticides, according to their immediate efficacy, rather than supporting the investigation of the potential for preventative actions, as recommended by the principles of IPM.

10. References

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