J. Farm Sci., 37(3): (233-237) 2024

RESEARCH PAPER

Quantification of field survival of *Pectinophora gossypiella* (Sanders) in *Bt* cotton across India and their resistance to cry toxins through diagnostic dose assays

SHANTHALA P. KARABASALLAVAR[1], *S. S. UDIKERI[1] AND R. S. BHAT[2]

[1]Department of Agricultural Entomology, [2]Department of Biotechnology, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad - 580 005, India

*E-mail: ssudikeri@gmail.com

(Received: November, 2020 ; Accepted: August,2024)

DOI: 10.61475/JFS.2024.v37i3.05

Abstract: The survival of pink bollworm *Pectinophora gossypeilla* (PBW) larvae in *Bt* cotton bolls is a serious concern. The field scale survival and associated *Bacillus thuringiensis* Cry toxin resistance has been quantified across 13 locations of India during 2018-19 representing major cotton growing areas. Damage was highest in Junagadh (Gujarath) with 0.89 boll occupancy index (BOI) and 91.42% infestation. Other locations having severe incidence were Surat (Gujarath), Guntur (Andhra Pradesh) Akola (Maharashtra), Coimbatore (Tamil Nadu) with BOI of 0.76, 0.72, 0.71, 0.58, respectively. With low BOI of 0.13 and 0.16 PBW field scale damage appeared to be least in Faridkot (Punjab) and Sirsa (Haryana), respectively. Further, resistance in field strains of these 13 places could also differ in degree of resistance to Cry 1Ac and Cry 2 Ab toxins. The sensitivity of PBW larvae from Junagadh was least with 62.5 ± 5.55% survival to diagnostic dose of 5 µl/mL of Cry1Ac toxin in 21 days test. The places with higher level of resistance to Cry 1Ac were Akola, Surat, Parbhani, with 55.0 ± 4.98, 50.0 ± 2.88, 52.5± 4.78 per cent PBW survival, respectively. Similarly, resistance to Cry 2 Ab toxin to diagnostic dose of 5 µl/mL diet was higher in PBW strains from Junagadh, Akola and Surat with and 55.0 ± 1.96, 52.5±1.00 and 45.0 ± 1.49 survival. Contrarily with low survival the strains of Faridkot and Sirsa appeared to be sensitive to both Cry 1 Ac and Cry 2Ab. Thus, with higher boll occupancy and lower sensitivity Central and South Indian cotton zones appeared to have alarming status and North Indian PBW strains just acquiring resistance.

Key words: Bollworm, Cotton, Cry toxin, *Pectinophora gossypiella,* Resistance

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the leading plant fiber crop being grown worldwide commercially in the temperate and tropical regions Cotton is cultivated in 77 countries, and among these China, India, United States, Brazil and Pakistan are major producers with 78 per cent of the total world production from 72 per cent of the world gross cotton area (Anon, 2019). Among the array of insects, especially the bollworms (Dhurua and Gujar 2011) *viz.*, American bollworm, *Helicoverpa armigera* (Hubner), spiny bollworm, *Earias insulana* (Boiusduval), spotted bollworm, *Earias vittella* (Fabricius) and pink bollworm, *Pectinophora gossypiella* (Saunders) normally referred as bollworm complex, pose greater threat to cotton production (Ghosh, 2001; Kranthi, 2015), in India. It caused major damage in many cotton growing countries, including China, India, USA and Pakistan. Yield loss estimates were 61 per cent in the USA (Schwartz *et al.,* 1983), 17-26 per cent in China (Luo *et al.,* 1986) and 20-30 per cent in Pakistan (Mallah *et al.,* 2000), 20.2 per cent in India (Agarwal and Katiyar, 1979).

The intensive use of chemical insecticides caused widespread ecological disruption leading to exacerbation of bollworms and secondary pest problems in cotton ecosystem (Kranthi *et al.,* 2002; Kranthi and Russell, 2009). In order to overcome this problem, genetically modified (GM) *Bacillus thuringiensis* var. *kurstaki* Berliner cotton (*Bt*-cotton) expressing a Crystal (Cry) protein Cry1Ac (*Bt*-1 cotton) derived from *B. thuringiensis* was introduced.

Adoption of *Bt*-cotton in India was very rapid (Chaudhary and Gaur, 2013). The area under BG-I cotton in India was 40.0 per cent in 2006 and 60 per cent in 2007, after which, BG I was progressively replaced by BG II and almost being phased out by 2014. The area under BG II increased from 46.2 per cent in 2009 to 91.0 per cent of the total cotton area in 2013. In the subsequent years after 2014, the area of *Bt*-cotton increased to 95-96 per cent of the cotton area in India, almost completely under BG II. Consequently, the acreage of *Bt* cotton has increased rapidly around the world because it was highly effective against its target insect pests and benign to the environment.

Bt cotton (BG) offered a high level of resistance against the cotton bollworm complex both in the laboratory as well as field conditions in India (Ghosh, 2001; Kranthi, 2002; Kranthi and Kranthi, 2004). Pink bollworm larval survival on BG-II was recorded significantly higher during 2012, 2013 and 2014 mainly in Amreli and Bhavnagar districts in Gujarath, while the damage ranged between 0 - 80 per cent on BG II in Bharuch Vadodara, Anand, Bhavnagar, Amreli, Junagadh, Rajkot, Surendranagar and Ahmedabad districts (Kranthi, 2015). The infestation of pink bollworm was observed up to 100 per cent in Vadodara and 14.05 per cent incidence in Kheda district in Gujarath state (CABI, 2017). The infestation of pink bollworm in Maharashtra state is ranged from 40 to 95 per cent (Fand *et al.,* 2019).

Understanding the status of pink bollworm resistance to *Bt* toxins is essential to develop strategies for its management. Hence, this investigation was carried out, to know the Cry toxins resistance in pink bollworm populations in India through elucidation of field infestation levels and survival of larvae to a diagnostic dose

Material and methods

The green bolls of cotton were collected from 13 different cotton growing regions of India. Among those seven cotton growing regions from South zone *viz.*, Raichur, Dharwad, Mysore, Haveri (Karnataka), Guntur (Andhra Pradesh), Coimbatore (Tamil Nadu) Warangal (Telangana) four regions in Central Zone, *viz.,* Akola, Parabhani (Maharashtra), Surat and Junagarh (Gujarat) and two regions in North Zone *viz.,* Faridkot (Punjab) and Sirsa (Haryana) to have proper representation from major cotton growing areas of the country. The bolls collection was from *Bt* cotton fields only in all these locations.

The bolls collected were brought to the entomology laboratory of Agricultural Research Station Dharwad, where the bioassays were conducted. Green bolls collected from field were split open to record the live (second in star onwards) pink bollworm larval incidence. Based on live larvae present in the bolls infestation (%) and pink bollworm occupancy index was calculated location wise and data presented (Table. 1).

$$\text{Boll occupancy index (BOI)} = \frac{\text{Number of pink bollworm larvae recovered}}{\text{Number of bolls screened}}$$

Green bolls collected from different locations were kept in ventilated cages at temperature of 29±1 ^{0}C, relative humidity 65 ± 5 per cent and 12 h photoperiod (Paul *et al.,* 1987). Infested bolls were cut open with a knife. The live larvae and pupae of pink bollworm recovered were counted location wise. Both the laboratory-reared susceptible culture and field collected cultures were maintained on an artificial diet. The diet and cultures were maintainance was as per Dhara Jothi *et al.* (2016) who recommended a cotton seed flour based diet.

The pupae were collected and washed with 0.2 per cent sodium hypochlorite for two minutes. Then the pupae were kept in plastic containers until adult emergence. The newly emerged adults were provided with 20 per cent honey solution + 1 ml multivitamin drop + Protinex and placed in oviposition chamber for egg laying in which 27±1 °C temperature and 65 ±5 per cent relative humidity were maintained. After hatching, the neonates were transferred to small plastic cups of 30 ml size containing approximately 5 g of artificial diet.

The diagnostic dose of both Cry toxins (5.0µg/mL of diet) was used for assessing sensitivity adapting diet incorporation method (Sims *et al*.,1996).The freeze-dried commercial formulation MVP-II® (Cell-Cap® encapsulation developed by Mycogen, San Diego, California, USA) was used as the source of Cry1Ac protein for different bioassays. MVP-II® is a lyophilized form of a liquid formulation containing Cry1Ac encapsulated in Pseudomonas fluorescence. This formulation contained 19.7 per cent (w/w) of Cry1Ac protein, as determined using the diet-incorporation method. A primary stock solution of Cry1Ac was prepared by vortexing 12.69 mg MVP-II powder in ten ml of 0.2 per cent agar solution. 5 gm/L of working solution made by dissolving 20 mL of primary stock in 1 L of 0.2 % agar solution. Similarly, source of Cry2Ab protein was leaf powder of transgenic maize plants (event MON 84 006) containing 3 mg Cry2Ab protein per 1 g of corn leaf powder. 5 gm/L of working solution made by dissolving 1.67gm of corn leaf powder in 1 L of distilled water.

Bioassay study to know the status of Cry1Ac and Cry2Ab toxins resistance in different populations of pink bollworm

Bioassays were carried out to know the response of pink bollworm larvae to a test dose 5µg/ml of each toxin as well as to know the exact level of resistance through probit analysis.

A 21 days bioassay was carried out using diet incorporation method (Sims *et al.,* 1996) and test dose of 5 µg/ml of each toxin independently. The neonates of F_1 population from each strain/ culture were used in this study. The larvae developing to at least third instars were recorded as live and underdeveloped larvae were considered as dead. The mortality up to 21 days in each culture was corrected with respect to untreated control (without toxin) as per Tabashnik *et al.* (2000). The number of larvae used per each treatment (toxin, strains) was 10 which has been replicated four times.

The mortality in the treated population was corrected concerning Abbott's formula (Abbott, 1987).

$$\text{Corrected mortality (\%)} = \frac{\text{(Percent test mortality - Percent control mprtality)}}{\text{(100- per cent control mortality)}} \times 100$$

Table 1. Status of pink bollworm field infestation in different *Bt* cotton growing regions of India

Cotton zones of India	Date	Place (State)	No of bolls collected	Infested bolls	Total no of larvae recovered	Infestation (%)	PBW boll occupancy index (BOI)
North zone	18/03/2019	Faridkot (Punjab)	79	28	10	35.44	0.13
	04/12/2018	Sirsa (Haryana)	370	151	60	40.81	0.16
Central zone	07/12/2018	Junagadh (Gujarath)	70	68	62	91.42	0.89
	22/11/2018	Surat (Gujarath)	85	75	65	88.23	0.76
	30/11/2018	Akola (Maharashtra)	45	39	32	86.66	0.71
	04/12/2018	Parbhani (Maharashra)	65	48	35	73.84	0.54
South zone	19/11/2018	Warangal (Telangana)	105	75	52	71.42	0.50
	03/12/2018	Guntur (Andhra Pradesh)	525	510	378	76.57	0.72
	06/02/2019	Coimbature (TamilNadu)	85	65	49	76.47	0.58
	13/11/2018	Dharwad (Karnataka)	130	95	70	73.07	0.54
	02/10/2018	Mysore (Karnataka)	199	120	68	60.30	0.34
	14/11/2018	Haveri (Karnataka)	195	125	85	64.13	0.44
	06/11/2018	Raichur (Karnataka)	189	110	69	58.20	0.37

Further, the resistance percentage was worked out using the formula.

$$\text{Resisitance (\%)} = \frac{\text{1-No. of dead larvae x 100}}{\text{No of larvae dosed}}$$

$$\text{Error (E)} = \frac{P\ (100 - P)}{(n-1)}$$

Where:

E= Binomial standard error

P = Percentage of larvae surviving discriminating dose

n = Total number of larvae tested that fortnight

Results and discussion

Studies on pink bollworm infestation at 13 different cotton-growing regions showed unusually high incidence at Junagadh (91.42%), Surat (88.23) in Gujarat followed by Akola (86.66%) and Parabhani (73.84%) in Maharashtra, of central India. In South India, Guntur (76.57%) Andhra Pradesh, Coimbatore (76.47%), Dharwad (73.07%) Karnataka, were having higher incidence. Similarly, in Karnataka (Shrilakshmi and Udikeri, 2021) Maharashtra (Jeughale *et al.,* 2007; Dhurua and Gujar, 2011) and in Gujarath (Kranthi, 2015) could observe severity of pink bollworm pink bollworm. The higher infestation of pink bollworm is an indication of its adoptability in BG-II and BG-I *Bt* cotton.

From the test dose analysis, it was evident that the pink bollworm population of Junagadh from Central India was having the highest resistance with survival of 62.5 ± 5.55 per cent, against Cry1Ac. Among the South Indian populations the Warangal population recorded highest percent survival of 47.5 ± 4. On the contrary in the North Indian population, Faridkot population showed only 10 ± 1.00 per cent of survival, followed by Sirsa 20 ± 1.96% which indicated low level of resistance. The similar observations were seen for Cry2Ab toxin also. The Junagadh population could show highest survival of 55 ± 1.96 per cent followed by South Indian populations. The highest survival was noticed in the Warangal population (37.5 ± 1.73%) in the South. On the contrary survival was low indicating a low level of resistance in North Indian population *viz* Sirsa 7.5 ± 0.75 per cent. followed by Faridkot 5 ± 0.50 per cent, (Table 2).

The studies conducted by Patin *et al. (*1999), Dennehy *et al. (*2005), Fabrick *et al. (*2014) and Ojha *et al. (*2014) have indicated survival of pink bollworm laboratory resistant strains to test doses. Further, Mohan *et al.* (2016) have subjected laboratory susceptible and field resistant strains where the susceptible and resistant population subjected to test doses of 1.0 and 10 µg/mL of Cry1Ac. The results of this study could clearly differentiate the field resistant strains of pink bollworm from susceptible strains in the mortality. Similarly, Dennehy *et al.* (2003) reported 98 and 100 per cent mortality of the pink bollworm populations at concentrations of 1.0 and 10 µg/ml, respectively, in Arizona collections.

Table 2. Response of pink bollworm populations for test dose of Cry toxins

Location	Survival (%) ± Binomial standard error	
	Cry1Ac	Cry2Ab
Susceptible (Lab.)	0	0
Faridkot (Punjab)	10.0 ± 1.00	5.0 ± 0.50
Sirsa (Haryana)	20.0 ± 1.96	7.5 ± 0.75
Surat (Gujarat)	50.0 ± 2.88	45.0 ± 1.49
Junagadh (Gujarat)	62.5 ± 5.55	55.0 ± 1.96
Akola (Maharashtra)	55.0 ± 4.98	52.5 ± 1.00
Parbhani (Maharashtra)	52.5 ± 4.78	40.0 ± 1.49
Warangal (Telangana)	47.5 ± 4.38	37.5 ± 1.73
Guntur (Andhra Pradesh)	37.5 ± 3.54	27.5 ± 1.24
Coimbatore (Tamil Nadu)	30.0 ± 2.88	25.0 ± 1.96
Dharwad (Karnataka)	32.5 ± 3.10	22.5 ± 1.49
Mysore (Karnataka)	25.0 ± 2.43	17.5 ± 2.20
Haveri (Karnataka)	30.0 ± 2.88	30.0 ± 1.00
Raichur (Karnataka)	27.5 ± 2.66	27.5 ± 1.49

Further, for Cry2Ab, the studies conducted by Dennehy *et al. (*2005), Mohan *et al.* (2010) and Malthankar and Gujar, (2015) have shown impact of the susceptible to test doses of 1 and 10 µg/mL on susceptible strain and resistant strains, where in only susceptible strains could show 90 to 100 per cent mortality against reduced mortality in resistant strains.

Thus, the fact that pink bollworm populations have gained resistance to the Cry toxin has been better confirmed through this study. Though the survival of pink bollworm in Bt genotypes was reported during 2008 (Dhurua and Gujar, 2011) from Gujarat, the resistance issues gained attention later. The previous studies have shown a considerable level of resistance to Cry1Ac in Gujarat and Maharashtra population (Fabrick *et al.,* 2014) for Cry2Ab (Malthankar and Gujar, 2015). Further, the findings of Naik *et al.* (2017) have a comprehensive report of resistance to Cry1Ac as well as Cry2Ab from 2010 to 2017. These findings indicate higher resistance in Central India followed by South India and least in North India.

The cause for less resistance or high sensitivity to Cry toxins in North Indian population could be the late adaptation of *Bt* transgenic cotton which directly started cultivation of BG II genotypes. Also the date of sowing is almost uniform hence flowering commences at a time which is not providing scope for continuous availability of *P. gossipiella* Further, cultivation of desi cotton and different sowing patterns could also be the reason for susceptibility. On the contrary in South India and Central India *Bt* cotton adaptation is from 2002 itself. Staggered showing pattern facilitates continuous flower availability. Thus, there is variation in the selection pressure offered by the *Bt* toxins. Further, it is advisable to adopt IPM strategies having bio control tools for management of pink bollworms as sugested by Udikeri *et al,* 2022

References

Abbott W S, 1987, A method of computing the effectiveness of an insecticide *Journal of the American Mosquito Control Association,* 3(2): 302-304.

Anonymous, 2019, ICAR-AICRP (Cotton) Annual Report (2018-19) ICAR – All India Coordinated Research Project on Cotton, Coimbatore - 641 003 www.cicr.org.in .

Agarwal R A and Katiyar K N, 1979, An estimate of losses of seed kapas and seed due to bollworms on cotton in India. *Indian Journal of Entomology,* 41(2): 143-148.

CABI, 2017, Invasive species compendium: *Pectinophora gossypiella* (pink bollworm). Available online at://www.cabi.org/isc/datasheet/39417#70AF7142-7A8 B-4F36-A0BA-4F14FA270EED, accessed on 21/11/2017.

Choudhary B and Gaur K, 2013, Biotech cotton in India: A Country Profile 2002 -2012. ISAAA Series of Biotech Crop Profiles (ISAAA, Ithaca, NY). www.isaaa.org

Dennehy T J, Shriver L, Sims M A, Holley D, Carriere Y, Abashnik B, Antilla L and Whitlow M, 2003, Susceptibility of Arizona pink bollworm to Cry1Ac following six years of intensive use of transgenic *Bt* cotton in Arizona. Cotton: College of Agriculture and Life Sciences Report, http: //cals. arizona. edu/ pubs/crops/az1312/az13125d.

Dennehy T J, Unnithan G C, Harpold V S, Carriere Y and Tabashnik B E., 2005, Susceptibility of pink bollworm to *Bt* toxins Cry 1Ac and Cry2Ab2 in the Southwestern USA in 2005. Univ. Arizona, College of Agriculture Cotton Report, P. 30.

Dhara Jothi B, Naik C B, Kranthi S, Kranthi K R and ValarmathiViable R, 2016, Mass production method for cotton pink bollworm, *Pectinophora gossypiella* (Saunders) *The Journal of Basic and Applied Zoology,* 73: 9-12.

Dhurua S and Gujar G T, 2011, Field evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. *Pest Management Science,* 67: 898-903.

Fabrick J A, Ponnuraj J, Singh A, Tanwar R K, Unnithan G C, Yelich A J, Li X, Carriere Y and Tabashnik B E, 2014, Alternative splicing and highly variable cadherin transcripts associated with field-evolved resistance of pink bollworm to *Bt* cotton in India. *Insect Biochemistry and Physiology,* 8: 112-127.

Fand B B, Nagrare V, Gawande S, Nagrale D, Naikwadi B, Deshmukh V, Narkhedkar G N and Waghmare V, 2019, Widespread infestation of pink bollworm, *Pectinophora gossypiella* (*Saunders*) (Lepidoptera: Gelechidae) on *Bt* cotton in Central India: A new threat and concerns for cotton production. *Phytoparasitica*, 47: 1-13.

Ghosh P K, 2001, ISCI Silver jubilee lecture series-lecture on genetically modified crops in India with special references to Bt-cotton. *Journal of Indian Society for Cotton Improvement*, 18(4): 106-107.

Jeughale G S, Kakade S U and Kadam S R, 2007, Effect of Bt cotton hybrids as one of the components of IPM on pink bollworm incidence under rainfed situation. *Crop Research*, 34: 206-209.

Kranthi K R and Kranthi N R, 2004, Modelling adaptability of cotton bollworm *Helicoverpa armigera* (Hubner) to *Bt*-cotton in India. *Current Science,* 87(8): 1096-1107.

Kranthi K R, 2015, Pink bollworm strikes *Bt* cotton. *Cotton Statistics and News*, 35(1): 1-6.

Kranthi K R and Russell D A, 2009, Changing trends in cotton pest management. In: Integrated pest management: Innovation-development (Ed. Peshin, R. and Dhawan, A. K.), Springer, 42: 499-541.

Kranthi K R, Russell D, Wanjari R, Kherde M, Munje S, Lavhe N and Armes N, 2002, In-season changes in resistance to insecticides in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in India. *Journal of Economic Entomology*, 95(1): 134-142.

Luo S B, Yan J P, Chai C J, Liang S P, Zhang Y M, Zhang Y and Le G K, 1986, Control of pink bollworm, *Pectinophora gossypiella* with *Bacillus thuringiensis* in cotton fields. *Chinese Journal of Biological Control*, 2(4): 167-169.

Mallah G H, Soomro A R, Soomro A W, Kourejo A K and Kalhoro A D, 2000, Studies on the left over standing cotton as carry-over sources of pink bollworm in Sindh. *Pakistan Journal of Biological Sciences,* 3(1): 147-149.

Malthankar V and Gujar S, 2015, Toxicity of *Bacillus thuringiensis* Cry2Ab and inheritance of Cry2Ab resistance in pink bollworm, *Pectinophora gossypiella* (Saunders). *Indian Journal of Experimental Biology*, 54: 586-596.

Mohan K S, Ravi K C, Suresh P J, Sumerford D and Head G P, 2016, Field resistance to the *Bacillus thuringiensis* protein Cry1Ac expressed in Bollgard (®) hybrid cotton in pink bollworm, *Pectinophora gossypiella* (Saunders) populations in India. *Pest Management Science*, 72(4): 738-746.

Mohan M, 2010, Pink bollworm resistance to GM cotton in India (subsequently revised) https: //monsanto.com/ company/ media/ statements/ pink-bollworm-resistance/, revised page accessed on November, 15, 2017.

Naik V C, Sujit K, Kranthi S, Satija U and Kranthi K R, 2017, Field evolved- resistance of pink bollworm*, Pectinophora gossipiella* (Saunders) to transgenic *Bt* Cotton expressing Cry1Ac and Cry2Ab in India. *Pest Management Science*, 4: 136-143.

Ojha A A, Sowjanya S K, Sachdev B, Rashmi M A, Ravi K C, Suresh P J, Mohan K S, Bhatnagar R K, 2014, Analysis of resistance to Cry1Ac in field-collected pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), *GM Crops Food,* 5(4): 280-286.

Patil B V, Bheemanna M, Hanchinal S G, Hosamani A C and Bansi A B, 2007, Status of pink bollworm, *Pectinophora gossypiella* (Saunders) on cotton at Raichur, Karnataka. *Journal of Cotton Research and Development*, 21: 224-226.

Patin A L, Dennehy T J, Sims M A, Tabashnik B E, Liu Y B, Antilla L, Gouge D, Henneberry T J and Staten R, 1999, Status of pink bollworm susceptibility to *Bt* in Arizona. In: Proceedings of Belt wide Cotton Conferences, National Cotton Council, Memphis, TN, 52: 991-996.

Paul A V N, Prasad B and Gautam R D, 1987, Artificial diet for *Pectinophora gossypiella* and *Earias vitella* bollworms of cotton. *Indian Journal of Agricultural Science*, 57: 89-92.

Schwartz P H, 1983, Losses in yield of cotton due to insects. Agriculture Handbook, USDA, No. 589: 329-358.

Sims S R, Greenplate J T, Stone T B, Caprio M A and Gould F L, 1996, Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins, molecular genetics and evolution of pesticide resistance. American Chemical Society Symposium Series 645. An American Chemical Society Publication, Washington, DC. 58: 230-242.

Shrilakshmi R G, Udikeri S S, 2021, Incidence of pink bollworm *Pectinophora gossypiella* Saunders in different agro- ecological zones of Karnataka. *Journal of Entomology and Zoology Studies,* 9(1): 607-612.

Tabashnik B E, Patin A L, Dennehy T J, Liu Y B, Carrière Y, Sims M and Antilla L, 2000, Frequency of resistance to *Bacillus thuringiensis* in field populations of pink bollworm. *Proceedings of National Academy of Science USA*, 97: 12980-12984.

Udikeri S S, Gundannavar K P, Hugar S V, Holeyannavar P, Nadaf Rajesh, 2022, Development and validation of an adaptable IPM module for pink bollworm in BG-II *Bt* transgenic cotton. 7th World Cotton Research Conference. 3-7,Oct 2022. Cairo, Egypt. Book of papers. Pp 477-485.