

**A move towards maximizing natural biological control of pink bollworm, *Pectinophora gossypiella* (saunders) (Lepidoptera: gelechiidae) in Sindh, Pakistan**

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ABSTRACT

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Continuous reduction in cotton production is seen in Pakistan due to arthropod infestations, especially pink bollworm, *Pectinophora gossypiella* (Saunders) that has become a major pest in recent years, is next to impossible to be controlled by insecticides. Consecutive use of insecticides in crops has weakened the natural biological control of pink bollworm (PBW), impacting crop yield. Parasitoids and predators are regarded as the eminent biological control agents of PBW worldwide. Rich natural enemies complex comprising 28 parasitoids species and 23 predators has been reported associated with PBW in Pakistan. A wealth of knowledge is available on indoor parasitoids rearing and manual release in fields, but little is known on mass multiplication of parasitoids in field conditions and their natural dispersion. The role of innovation in establishing outdoor facility to say natural enemies' field reservoir (NEFR), was tested for mass production of parasitoids and their dispersion naturally, to maximize natural biological control of PBW in District Sanghar. Twelve NEFR sheds/shelters, open from all sides, were built at variable distances of 5-10 km from one another to cover 2400 acres of cotton crop. At shelters PBW infested flowers and bolls were kept for 15-20 days in zincoid trays and transparent plastic jars, covered with a net of adequate hole size that allowed easy escape of parasitoids and held back the PBW moths. To start with, observations were made in March-June 2021 to confirm the presence/absence of PBW parasitoids in flowers and bolls, whereas from July onwards, not only activities were confined to mass production of parasitoids but also determined the parasitism rate in PBW for indirect estimates of parasitoids dispersing in the environment. Large collection of PBW larvae (about 12 million) was also made in November and December from ginning mills refuse (cotton seeds and fibers), and kept in trays at 12 NEFR shelters. Most of parasitoids recorded were solitary but some of them were gregarious, such as *Bracon gelechia*. Variable parasitism rates were observed in PBW larvae and pupae collected from different sources being highest (43%) in rosette blooms followed by cotton bolls (31%) and ginning wastes (23%). Out of 7.8 million larvae and pupae collected in July- November from green and harvested crop wastes, were obtained 2.1 million parasitoids. More advantageous of NEFR was of eradication of 5.7 million PBW moths (males and females) from cotton environment. Thus, besides the conservation of parasitoids, millions of PBW adults were destroyed which probably was not possible with the pesticide sprays in the field. Observations on slow emergence of pests and parasitoids from mills refuse continued through February 2022. These findings are food for thought to move forward with the promotion of NEFR-like resource conserving technologies for sustainable management of insect pests. Furthermore, our study presents an innovative and eco-friendly approach for managing pink bollworm through the establishment of Natural Enemies' Field Reservoirs (NEFRs). This field-based strategy effectively promoted the mass multiplication and natural dispersal of parasitoids, resulting in the production of over 2.1 million parasitoids and the eradication of approximately 5.7 million PBW moths. Covering 2400 acres with 12 shelters, the study demonstrated the large-scale applicability and sustainability of NEFRs. Using naturally infested cotton flowers, bolls, and ginning waste provided a cost-effective and resource-efficient method, significantly reducing pesticide dependence and enhancing natural biological control within the cotton ecosystem.

Keywords: Natural enemies field reservoir, NEFR, cotton, pink bollworm, *bracon gelechia*, cotton, sanghar.

INTRODUCTION: Cotton (*Gossypium hirsutum* L.) is known as most important crop across the world which provides raw material to fiber/textile industry, edible oil and revenue by exports (Ozyigit *et al.*, 2007). Pakistan which is the 5th largest producer of cotton (Survey by M. Shahbandeh, Statista), has cultivation of cotton crop on largest area compared to rest of the crops (Economic Survey of Pakistan), contributing 55% to exports (earning of foreign exchange) and 1% to gross domestic product (GDP) (Rehman *et al.*, 2019). Over the past decade, cotton production in Pakistan is continuously presenting declining trend due to acute attack of arthropod pests, injudicious use of pesticide and fertilizers, occurrence & prevalence of pathogenic micro-flora, and abiotic stress such as rise in temperature and CO₂ concentrations, prolonged drought spells (Rehman *et al.*, 2019).

Arthropod infestation is known to cause major damage to the cotton crop which may reduce 35-45% yield (Haq, 1991; Kannan *et al.*, 2004). Variety of insect pests (sucking and bollworms complexes) victimize cotton crop across the world (Abdullah, 2010), but Pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) alone is considered to be most devastating insect pest and showed ineffectiveness of chemical control due to concealed feeding behavior (Lykouressis *et al.*, 2005). *P. gossypiella* has shown to cause loss of 20-30% bolls (Khuhro *et al.*, 2015). Adults of *P. gossypiella* can live 10 days to 2 weeks in midsummer (longer in cold weather) and lay about 130-200 eggs on all parts of the cotton plant, commonly on fruiting forms (i.e. squares, stems, bolls, terminal buds). Larvae take very short period of time to hatch out and enter into the fruiting forms within half an hour, a very short time of exposure to natural enemies (Noble, 1969). These larvae feed on fruiting forms, resulting abnormality in opening of bolls, lowered quality of lint (discoloration and reduction in the length and strength of fiber), introduction of boll-rot organisms via larval exit holes, and reduction of weight/yield (Hutchison *et al.*, 1988). *P. gossypiella* completes development of first 3-4 generations on cotton crop in summer, whereas larvae of next generations enter into diapause for overwintering in leftover bolls (after last picking

of cotton), as well as seeds/refuse of cotton ginning factories (Ahmed, 2013).

Over the decades, multiple integrated pest management strategies such as cultural control, chemical control, host plant resistance (*Bt*-cotton), sterile male release, mating disruption through pheromone-baited moth traps etc., were developed and exercised to control/eradicate *P. gossypiella*, that showed significant results for some periods of time, but long-term solution of this devastating insect pest has not yet developed. The impact of Biological control via natural enemies especially parasitoids with least application of chemicals converged significant attention of scientists/entomologists in recent years. Among IPM practices, natural enemies have been increasingly recognized to control cotton pests especially pink bollworms in different parts of the world, that not only minimized application of pesticides (El-Heneidy *et al.*, 1987), but also provided environment friendly and an economical ways of pest management.

Parasitoids are most eminent biological control agents among natural enemies of *P. gossypiella*, that are amenable to mass-rearing and production to reduce the damage caused by *P. gossypiella* (Duny *et al.*, 1998), cost effective (at least 65% reduction in protection cost), and comparatively efficient to suppress pest populations (Kogan, 1998; Khidr *et al.*, 2003). In Pakistan, (Cheema *et al.*, 1980a,b) reported naturally occurring of 28 parasitoid species and 23 species of predators from cotton fields which provide evidence of sufficient parasitic fauna, as well as an excellent scenario to control insect pest of cotton crop through mass-rearing/production and release of biological control agents like parasitoids. A wealth of knowledge is available on parasitoid rearing/multiplication under laboratory conditions (Ahmed *et al.*, 2017; Argov *et al.*, 1998; Bertin *et al.*, 2017; Canale *et al.*, 2012; Harris and Richard, 1991), but rearing/multiplication and dispersion of parasitoids in natural conditions are yet to be explored. In our present study, we discussed biological control of *P. gossypiella* via parasitoids under natural conditions by establishing natural enemies field reservoirs (NEFRs) in the cotton fields, following an innovative hypothesis to boost populations of natural enemies against their potential hosts,

collecting *P. gossypiella* individuals mechanically from different sources and analyze their parasitism rates. For our study, District Sanghar (Province Sindh, Pakistan) which is known as an important regime of cotton cultivation and hub of cotton ginning factories, provided an excellent scenario to analyze performance of NEFR Technology.

MATERIALS AND METHODS: Establishment of natural enemies field reservoirs (NEFR): Twelve open shelters/NEFRs (each shelter size: 20'x40'x10') having a small attached room (for laboratory purpose) at one corner, constructed in cotton fields of District Sanghar, Province Sindh, Pakistan (figure 1), for protection and mass production of parasitoids that attack *P. gossypiella* individuals. Under each shelter, 10 zincoid metal boxes (4'x2'x2'), and 16 zincoid metal trays (3'x1.5'x8") were placed to sustain populations of *P. gossypiella* on culture allowing them for rapid multiplication and consequent dispersal in the surrounding cotton fields. Each NEFR aimed to benefit the radius of 200 acres of cotton cultivated land.

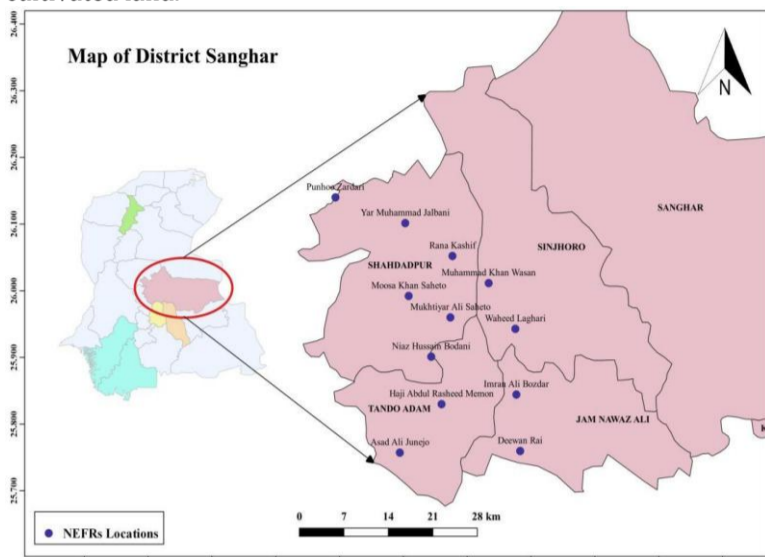


Figure 1: Twelve Natural Enemies Field Reservoirs (NEFRs) located at Village Asad Ali Junejo/Nadir Farm (25°45'30.2"N 68°37'52.3"E), Haji Abdul Rasheed Memon/Wahab Farm (25°48'02.3"N 68°39'15.4"E), Village Dewan Rai (25°47'05.0"N 68°48'22.0"E), Village Imran Ali Bozdar (25°50'50.5"N 68°45'44.1"E), Village Niaz Hussain Bodani (25°47'39.3"N 68°40'28.2"E), Village Waheed Laghari/Lal Khan Laghari (25°57'21.4"N 68°44'45.8"E), Village Mukhtiyar Saheto/Asgharabad (25°58'40.7"N 68°40'01.0"E), Village Moosa Khan Saheto/Nabi bux Saheto (25°58'58.3"N 68°39'54.5"E), Village Muhammad Khan Wasan/Barhon (26°03'33.2"N 68°42'21.8"E), Village Rana Kashif (26°04'31.3"N 68°43'51.3"E), Yar Muhammad Jalbani (26°05'26.3"N 68°42'51.4"E), and Village Punhoon Zardari/Imam Bux Sanjrani (26°05'47.3"N 68°42'45.4"E) in District Sanghar-Sindh.

Collection of *P. gossypiella* larvae from different sources: Adequate presence of *P. gossypiella* was observed in rosette blooms of cotton infested by *P. gossypiella*. These were collected from the cotton fields mechanically and placed them into the open basket (2'x1') (figure 2).



Figure 2: Pink bollworm (*Pectinophora gossypiella*) infested flowers (rosette blooms) collection for NEFRs. These flowers were kept under the NEFR shelter for couple of days in order to attract parasitoids as well as to dry for minimizing

chance of fungal attack. These infested flowers and 1000 *P. gossypiella* larvae were then kept individually in plastic jars (1'x6") for 15 days to observe emergence of moths and parasitoids. These plastic jars were modified (windowed) for ventilation to avoid fungal attack on flowers and consequent death of larvae. These windows were properly covered with mesh for easy escape of newly emerged parasitoids in the cotton fields and to hold moths inside till death. Same mesh was used to cover open mouths of jars. Emergence of moths/parasitoids and dispersal of parasitoids from the jars were observed daily. Unopened infested cotton bolls were collected from cotton fields of NEFRs surrounding, and transported them to NEFR shelters (figure 3). For NEFR purposes, larvae were collected from cotton bolls by opening them and carefully placed in the plastic jars (1000 larvae/jar) for 15 days. Moist cotton with seed (pulp of unopened cotton boll) was used as natural diet for *P. gossypiella* individuals. Emergence of moths/parasitoids and dispersal of parasitoids from the jars were observed.



Figure 3: Infested cotton bolls (leftover bolls) by Pink bollworm (*Pectinophora gossypiella*) collected from the cotton fields of District Sanghar for NEFRs.

Collection of *P. gossypiella* was carried out from different cotton ginning factories of District Sanghar. The refuse of cotton ginning factories was carefully transported to all NEFR shelters. Larvae separated from refuse and placed in the plastic jars (1000 larvae/jar) for 15 days. Moist cotton seed (pulp of unopened cotton boll) was used as natural diet for *P. gossypiella* individuals. For month-wise estimation of parasitoid dispersion, parasitism rate for each month was recorded by sampling 50 larvae available *P. gossypiella* collection at each NEFR shelter (600 larvae at all 12 NEFRs each month). These individuals were placed in small plastic transparent jars (3.5"x2.5") till emergence of moth/parasitoid(s). Numbers of newly emerged parasitoids/moth were recorded on daily basis.

In addition, huge collection of *P. gossypiella* larvae from cotton ginning factories was carried out at the end of the cotton season and transported to the NEFR shelters, and placed into the zincoid metal boxes (figure 4).



Figure 4: Refuse of Cotton ginning factories, placed in metal boxes under NEFR facility during cotton season for augmentation/strengthening populations of naturally occurring parasitoids of Pink bollworm (*Pectinophora gossypiella*). These boxes were covered by mesh for escape of parasitoids from boxes but to hold back the moths in boxes. Emergence of moths held

back in boxes and escape of parasitoids and dispersing in field around were observed to have idea of synchronization of parasitoids phenology with overwintering *P. gossypiella* populations and conservation of parasitic-fauna which get lost by unavoidable agricultural and social activities.

Parasitism: This experiment was aimed to explore potential of parasitoids in *P. gossypiella* individuals, collected from different sources. For this experiment, sample size was extended up to 1200 *P. gossypiella* larvae (100 larvae at each NEFR) from each source, kept separately in small plastic jars (3.5"x2.5") till emergence of moth or parasitoids. These jars were placed in a cluster of 50 jars per basket under natural conditions to observe parasitism percentage. Numbers of newly emerged parasitoids/moth were recorded on daily basis. Similarly, samples of 1200 *P. gossypiella* larvae (from rosette blooms) were also collected from different non-NEFR cotton crop fields for comparison of parasitism with the NEFR farms.

Impact of NEFR technology: Cotton production per acre of all surrounding cotton crop fields of 12 NEFRs was compared with production of previous year (NEFR cotton crop fields) and current production of non-NEFR cotton crop fields by conducting interviews of growers and previous records maintained by their managers/workers. Similarly, numbers of pesticide spray frequencies in surroundings of 12 NEFRs were also recorded and compared with previous year pesticide spray frequencies (before establishment of NEFR), and current spray frequencies of non-NEFR cotton crop fields to explore effect of NEFR Technology.

Statistical analysis: Analyses of variance (ANOVAs) in SPSS (IBM® SPSS® Statistics 20) were used to identify the significant differences among all comparisons. In case of overall variation in ANOVAs was significant, Tukey tests at $\alpha = 0.05$ was used for separation of means. To meet the required normality and homoscedasticity in ANOVAs, we conducted log-transformation of data (if needed).

RESULTS: From collection of about 7.8 million of *P. gossypiella* larvae, 2.1 million estimated parasitoids dispersed (based on determination of rate of parasitism percent in larvae), and about 5.7 million of estimated PBW moths at all NEFRs were recorded eliminated. Significant difference between September, October and November observed that reflects source effect (table 1). Collection that carried out during months of October and November, was higher than those of July to September, whereas collection from July to September showed increasing trend with the increase of infestation. Similar patterns were found in estimated parasitoids dispersal (month wise), and elimination of newly emerged caged moths. Our results showed increasing trend of parasitoid dispersion with the increase of *P. gossypiella* collection. Furthermore, *P. gossypiella* individuals of rosette blooms tended to have higher parasitism rates with increasing trend from July to September, compared to individuals of infested cotton bolls from cotton fields, leftover cotton bolls (after last picking of cotton from fields), and cotton ginning factories.

Parasitism: *P. gossypiella* individuals collected from rosette blooms tended to have higher parasitism rates in comparison to the individuals collected from other sources. *P. gossypiella* individuals of rosette blooms showed significantly higher parasitism than those of cotton ginning factories (figure 5; $F = 5.139$; $df = 3,36$; $P < 0.01$), and non-NEFR cotton crop field ($F = 5.139$; $df = 3,36$; $P < 0.02$). *P. gossypiella* individuals of cotton ginning factories, non-NEFR cotton crop fields and non-NEFR cotton crop fields showed parasitism rates with a slight difference but not significantly different from each other.

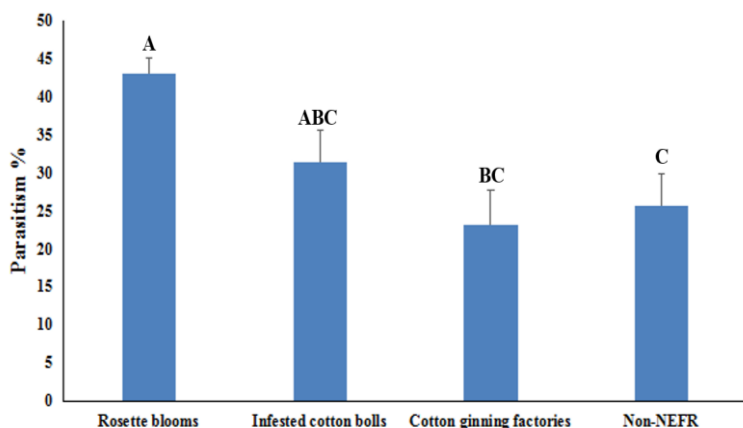


Figure 5: Parasitism (SE) in *Pectinophora gossypiella* collected from rosette blooms (Cotton crop fields with NEFR), infested cotton bolls

(Cotton crop fields with NEFR), cotton ginning factories (Non-NEFR), and Non-NEFR cotton crop fields. However, individuals of harvested cotton bolls showed higher parasitism rates than individuals of cotton ginning factories as well as non-NEFR cotton crop fields.

About 28 types of parasitoids associated with PBW have been reported from Pakistan (Cheema *et al.*, 1980a and 1980b), however, in present study, only five types were recorded (Figure 6). These have been sent to Natural History Museum UK for proper identification. Main reason of rarity of the parasitic fauna at District Sanghar seems to be due to adverse effect of excessive pesticides use in the cotton crop environment.



Figure 6: Five different naturally occurring parasitoid species found in surrounding cotton fields of twelve NEFR facilities at District Sanghar, Sindh Province, Pakistan.

Bracon gelechi, one of the five types is gregarious. Number of parasitoids emerged from single *P. gossypiella* individual of rosette blooms was significantly higher than those of collected from rest of the sources (figure 7; $F = 9.53$; $df = 3, 36$; $P < 0.001$). There was no significant difference found in the number of parasitoids emerged from *P. gossypiella* individuals of infested cotton bolls, cotton ginning factories and non-NEFR cotton crop fields.

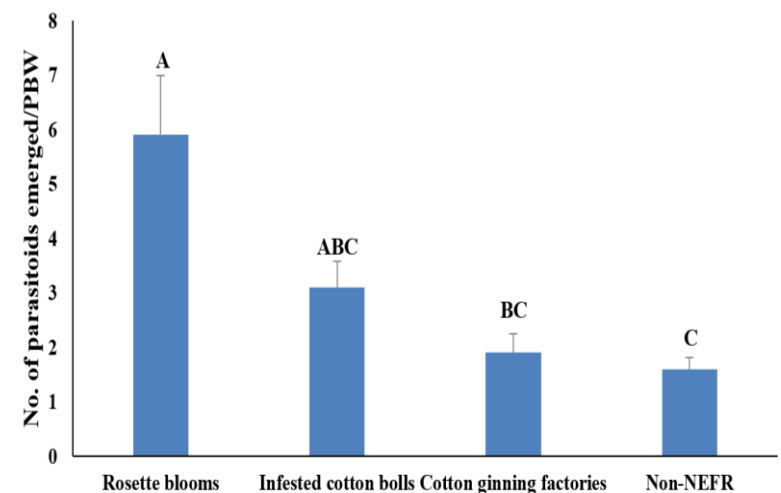


Figure 7: Number of parasitoids emerged from single *Pectinophora gossypiella* larva collected from rosette blooms (Cotton crop fields with NEFR), infested cotton bolls (Cotton crop fields with NEFR), cotton ginning factories (Non-NEFR), and Non-NEFR cotton crop fields.

Our results showed lower pesticide spray frequencies applied in cotton fields with NEFR than previous year records (before establishment of NEFRs), and non-NEFR (current season) cotton fields. We found significantly lower frequencies of pesticide spray in cotton fields around NEFRs (figure 8) than previous year and non-NEFR cotton fields. No significant difference found among previous year records (cotton fields of NEFRs) and non-NEFR cotton fields (current season).

Additional results revealed clear temporal and spatial variations in parasitism and PBW population across the study period. Monthly data indicated an increasing trend in parasitism rates from July to November, strongly correlating with rising PBW infestations.

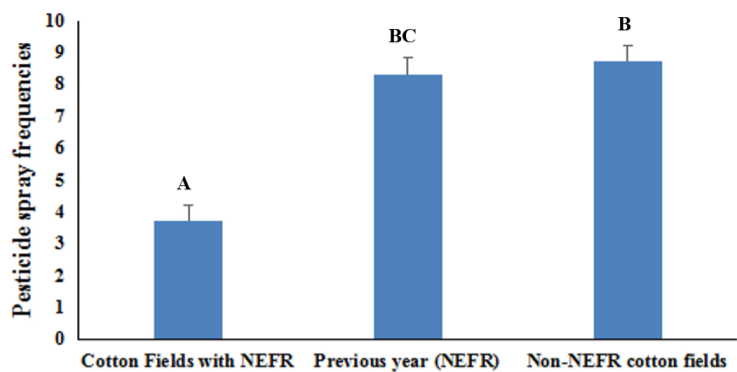


Figure 8: Comparison of mean pesticide spray frequencies (SE) on cotton crop fields around 12 NEFRs, previous year pesticide application (before establishment of NEFR), and Non-NEFR cotton crop fields (current season).

Among parasitoid species, *Bracon gelechia* was dominant, indicating a diverse natural enemy complex. Spatial analysis showed

higher parasitoid emergence near ginning areas and heavily infested fields, suggesting efficient natural dispersal from NEFRs. Environmental data indicated that parasitism peaked at moderate temperatures (32–36°C) and 60–70% relative humidity. An estimated 43% parasitism rate in rosette blooms demonstrated the superior host suitability of early-season infestations. NEFR efficiency was reflected in the production of 2.1 million parasitoids from 7.8 million PBW larvae, reducing the need for pesticide sprays and preventing 5.7 million moths from emerging, highlighting NEFRs as a cost-effective, sustainable, and ecologically viable pest management approach.

We found no significant difference among cotton production per acre of cotton crop fields around NEFRs, previous year (before establishment of NEFRs), and non-NEFR cotton crop fields (current season). However, we found slight increase in production per acre in the cotton fields of NEFRs.

Note: Different capital letters on bars indicate significant differences among pesticide spray frequencies at the $P < 0.05$ level, ANOVA followed by Tukey tests

Months	Collection of Pink bollworm Larvae	Rate of Parasitism	of Estimated dispersal of parasitoids	Estimated elimination of moths	Sources
July	42,200	34%	14,442	27,758	Rosette bloom
August	112,000	39%	44,200	67,800	Rosette bloom
September	277,000	49%	135,000	142,000	Rosette bloom
October	150,000	38%	56,300	93,700	Infested cotton bolls
November	7,250,500	26%	1,853,000	5,397,500	Leftover cotton bolls from fields & refuse of cotton ginning factories
Total	7,831,700		2,102,942	5,728,758	

Table 1: Month-wise collection of *P. gossypiella* individuals from different sources, parasitism rates, estimated dispersal of parasitoids, and estimated elimination of moths from July to November, 2021.

DISCUSSION: To control cotton pests especially pink bollworms, growers are dependent upon application of chemical pesticides (Mohamed *et al.*, 2010). However, protective feeding behavior of pink bollworms and development of resistance against chemical pesticides highlighted the need of alternative tools in which role of natural enemies like parasitoids, converged special attention in recent years. Naturally occurring parasitoids have significant potential of rapid multiplication and dispersion abilities with remarkable searching capabilities (Ahmed *et al.*, 2017). Our results presented that larvae of *P. gossypiella* collected from rosette blooms from cotton fields with NEFRs tended to have higher parasitism rates than individuals collected from cotton bolls, cotton ginning factories and non-NEFR cotton fields. One explanation of our results is clearly directing towards accessible/exposed hosts at the habitat of hosts (infested flowers). Off course, this might be regulated via chemical cues for parasitoids to search/locate their potential hosts, but larvae of *P. gossypiella* were easily accessible by the parasitoids in rosette blooms compared to those who bored cotton bolls and covered up entrance hole by their excreta. Similarly, individuals collected from cotton ginning factories were, in fact, belonged to opened cotton bolls/cotton seeds picked with raw cotton and brought to the cotton ginning factories. During whole process (from picking to ginning), there are some certain factors which might have effect on larvae of *P. gossypiella* such as injuries during cotton ginning process, exposure of sunlight/heat for long time before and after cotton ginning process, starvation etc.

P. gossypiella individuals collected from non-NEFR cotton fields showed lowest parasitism rates probably due to excessive use of pesticides that disrupted parasitoid populations in the cotton field which is also documented by (Atwal, 1994). In addition, consequent effect was observed in number of parasitoids from single larva of *P. gossypiella*, collected from rosette blooms which showed higher number of parasitoids emergence than those of collected from rest of the sources. This effect clearly denoting towards strong semiochemical attraction which provides cues to for parasitoids to access/locate the target larvae of *P. gossypiella*.

Our results showed higher/bumper collection of pink bollworms during months of October and November, carried out from leftover infested cotton bolls and cotton ginning factories, compared to collection of rosette blooms from cotton fields during whole season, showing significant effect of sources. Increase or decrease in collection of *P. gossypiella* individuals depended upon intensity of infestation and sources. *P. gossypiella* individuals were collected in bulk from cotton ginning factories and leftover cotton bolls, whereas rosette blooms were collected from the cotton fields individually, depended upon attack of *P. gossypiella* on cotton fields.

One of the objectives to develop NEFR Technology was to reduce application of pesticides in order to maximize and conserve populations of natural enemies in the cotton fields (Mahmood *et al.*, 2011, 2018 & 2019). Our results presented significant reduction in applications of pesticides from cotton fields with NEFR, compared to previous year records (before NEFRs) and non-NEFR cotton fields. These results support NEFR innovation as an alternative *P. gossypiella* management tactic instead of pesticides/insecticides application.

Positive impact of NEFR integration was also visible on per acre yield compared to yields of previous year and non-NEFR cotton fields (current season). Although, a significant increased level of productivity was not achieved in first season but, consistent production without application of pesticides reduced input costs that can be added as profit/productivity. Through NEFRs, not only conservation of the parasitoids was done, but also destruction of millions of males and females of *P. gossypiella* was an additional advantage that probably could not be achieved by use of pesticides in field. Moreover, consistent production without application of traditional control tactics like chemical control, is an achievement of NEFR Technology, that not only successfully replaced all previous practices to suppress population of pink bollworms within a short period of time, but also demonstrated as an environment friendly, economical, and sustainable pest control mechanism which could be acceptable to the growers. These findings are food for thought to move forward for further improvement of the NEFR innovation and promotion in the country and abroad.

CONCLUSION: This study demonstrates that the NEFR technology is an effective, environmentally sound, and sustainable approach for the management of pink bollworm (*Pectinophora gossypiella*) in cotton agro-ecosystems. Large-scale collection of PBW larvae resulted in the successful dispersal of an estimated 2.1 million parasitoids and elimination of approximately 5.7 million potential moths, clearly indicating the biological suppression potential of NEFRs. Parasitism rates increased consistently with rising PBW infestation, showing a strong density-dependent response of parasitoids. Among all sources, PBW larvae collected from rosette blooms exhibited significantly higher parasitism rates and greater emergence of parasitoids per larva, highlighting rosette blooms as the most suitable and accessible host habitat for parasitoid activity. In contrast, larvae collected from non-NEFR cotton fields showed the lowest parasitism, most likely due to intensive pesticide use that disrupted natural enemy populations. The limited diversity of parasitoids recorded in the study area further reflects the negative impacts of excessive chemical pesticide application on beneficial insect fauna.

Seasonal variation played a critical role, with higher PBW collections, parasitoid dispersal, and moth elimination observed during October and November, particularly from leftover cotton bolls and ginning factories. These sources proved valuable for mass recovery of PBW larvae and subsequent augmentation of parasitoid populations through NEFRs. The dominance of *Bracon gelechiae* among the recorded parasitoids suggests its key role in PBW regulation under NEFR-based management. Importantly, integration of NEFRs significantly reduced pesticide spray frequencies without causing any yield penalty. Cotton productivity remained stable, with a slight increase in NEFR fields, while reduced pesticide inputs lowered production costs and enhanced net economic returns. Beyond yield, NEFRs contributed to the conservation of natural enemies and prevented the emergence of millions of PBW moths, an outcome difficult to achieve through chemical control alone. Overall, the findings confirm that NEFR technology not only replaces conventional pesticide-dependent practices but also offers a cost-effective, ecologically viable, and farmer-acceptable solution for long-term management of pink bollworm. The study provides strong evidence to support wider adoption, further refinement, and scaling-up of NEFRs in Pakistan and other cotton-growing regions for sustainable pest management.

Declaration: We hereby certify that the current study has neither been published elsewhere, nor is it under consideration elsewhere, and will not be submitted elsewhere.

Certify from authors and co-authors: We certify that all co-authors have been informed and agree with the submission of this paper.

Ethics approval: Not needed for this study.

Consent for publications: Not applicable.

Conflict of interest: The authors declare that they do not have any personal relationships that can affect the work reported in this review.

REFERENCES: Abdullah, A. 2010. An Analysis of Bt. cotton cultivation in Punjab, Pakistan using the Agriculture Decision Support System (ADSS). *AgBioForum*, 3: 274–287.

Ahmed, Z. 2013. Model farming: saving cotton from Pink bollworm. Available online at: <https://www.pakissan.com/english/advisory/saving.cotton.frm.the.pink.bollworm.shtml>

Ahmed, S. S., D. G. Liu & J. C. Simon. 2017. Impact of water-deficit stress on tritrophic interactions in a wheat-aphid-parasitoid system. *PLoS ONE*, 12(10): e0186599.

Atwal. 1994. Pests of cotton. In: *Agricultural Pests of India and South East Asia*, Kalyani Publishers, Delhi, pp. 281–294.

Bertin, A., V. A. C. Pavinato & J. R. Postali Parra. 2017. Fitness-related changes in laboratory populations of the egg parasitoid *Trichogramma galloi* and the implications of rearing on factitious hosts. *BioControl*, 62(4): 435–444.

Cheema, M. A., N. Muzaffar & M. A. Ghani. (1980). Investigations on phenology, distribution, host-range and evaluation of predators of *Pectinophora gossypiella* (Saunders) in Pakistan. *Pakistan Cottons*, 24: 139–176.

Kannan et al. 2004. Impact of insecticides on sucking pests and natural-enemy complex of transgenic cotton. *Current Science*, 86(5): 726–729.

Khuhiro, S. N., F. Ahmad, A. M. Kalroo, K. Abdullah & M. A. Talpur. 2015. Monitoring and population dynamics of pink bollworm on cotton in different cotton-growing areas of Sindh, Pakistan. *International Journal of Interdisciplinary Research in Science, Society and Culture*, 1(2): 51–60.

Khidr et al. 2003. Comparative studies between the efficiencies of egg parasitoids, *Trichogramma evanescens* West., and the insecticidal applications against the cotton bollworms in Egyptian cotton fields. In: Proceedings of the 1st International Egyptian–Romanian Conference, Zagazig, Egypt, December 6–8, 2003.

Lykouressis, D. P., A. A. Economou & C. P. Tsitsipis. 2005. Management of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) by mating-disruption in cotton fields. *Crop Protection*, 24: 177–183.

Mahmood et al. 2019. Impact of augmentation of parasitoid *Acerophagus papayae* Noyes & Schauff (Hymenoptera: Braconidae) on papaya mealybug *Paracoccus marginatus* William Granara de Willink (Hemiptera: Pseudococcidae) in Karachi — Sindh. *FUUAST Journal of Biology*, 9(1): 157–162.

Mohamed, E. M., H. F. Abdel-Hafez & M. A. Abd-Aziz. 2010. Effect of adding some chemical additives on increasing the potency and residual effect of *Bacillus thuringiensis* against the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Egyptian Journal of Agricultural Research*, 88(1): 103–112.

Ozyigit et al. 2007. Relation between explant age, total phenols and regeneration response in tissue-cultured cotton (*Gossypium hirsutum* L.). *African Journal of Biotechnology*, 6: 3–8.



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