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RATIONAL MANAGEMENT OF *PHENOCOCCUS SOLENOPSIS* THROUGH CONSERVATION AND AUGMENTATION OF *AENASIUS BAMBAWALEI* AND *CRYPTOLAEMUS MONTROUZIERI* IN PAKISTAN

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ABSTRACT

Cotton mealybug (*Phenococcus solenopsis* Tinsley 1898) is one of the major sucking insect pest of cotton in Pakistan. This study was carried out during 2012 & 2013 in Multan District, Punjab, Pakistan to evaluate the response and conservation of biocontrol agents i.e. *Aenasius bambawalei* Hayat (= *A. arizonensis* Fallahzadeh, 2014) and *Cryptolaemous montrouzieri* Mulsant, 1850 against cotton mealybug (CMB). Biological responses of these biocontrol agents were assessed at different temperatures and humidity levels in the laboratory; which helped to design the rearing technologies for field releases. Data was collected at 100, 200 and 400 meters from NEFR surroundings after 07, 15 and 22 days of releases (DAR) of biocontrol agents. Results showed a significant decrease in CMB population; at 100 meter as 46%, 69% and 94% respectively; at 200 meter as 32%, 53% and 83% respectively; at 400 meter as 25%, 45% and 77% respectively. Cost benefit ratio of NEFR assessed was 1:1.8 and 1:3.2 when the crop losses reduced 20 to 50 percent in comparison with control field.

Keywords: Biological control, NEFR, natural enemies, population dynamics
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INTRODUCTION

Cotton (*Gossypium hirsutum*) is one of the major cash crop in Pakistan and contributes about 10% in GDP (Economic Survey of Pakistan, 2015-16). It undergoes pest pressure during its seasonal cycle and faces reduced production imposing economic implications to the Pakistan. Cotton mealybug (*Phenococcus solenopsis*) is among the major insect pest of cotton which sucks cell sap and affects plant growth in multiple ways. Initially, leaves turn yellow, crinkle and curly leading towards drop off. Infested flowers often drop and sometimes produce fewer deformed bolls or no fruit. (Yonas *et al*., 2019). Severely damaged plants suffer premature dehydration and give the appearance of defoliator spray on cotton plant. Afterwards, plant becomes stunted and death occurs. Under indirect infestation, it produces honeydew resulting in growth of sooty mold on leaves that hinders photosynthesis (Mark and Gullan, 2005; Saeed *et al*., 2007).

In Pakistan tropical and subtropical climate with summer rains and short winter favours mealybug breeding to keep it active throughout the year. All stages are evident with many overlapping generations found damaging plant parts such as leaves, shoots, twigs, branches, flowers and roots. Infestation occurs in patches that make huge loss to cotton yield (Mahmood *et al*., 2011).

Farmers in developing countries including Pakistan solely relies on application of chemical sprays to get immediate results for its management (Joshi *et al*., 2010). In addition, non judicial use of toxic pesticides impose environment and health implications due to their residual behavior and entry into food chain. There is need to evaluate and adopt some alternate management approach to manage cotton mealybug below economic threshold level. In this regard, exploration and utilization of biocontrol agents associated with cotton mealybug has received considerable attention all over the world. However, this concept is not familiar in developing countries due to lack of technical capacity and awareness among farming communities. Furthermore, biological control requires extensive identification and exploration of biocontrol agents and then designing their rearing and release technologies for rapid adoption at farm level.

Chrysoperla carnea and Coccinellid beetles are reported promising biocontrol agents to regulate population of cotton mealybug below economic threshold level (Agarwal *et al*., 1988; Gautam, 1996; Ali *et al*., 2014). Coccinellid beetles such as *Cryptolaemus montrouzieri* (known as mealybug destroyer), *Brumoides suturalis, C. septumpunctata*, *Hippodamia variegata*, *Menochilus sexmaculatus* and *Hyperaspis maindron*, are considered great destroyers of all stages of mealybugs (Ali *et al*., 2014). Among parasitoids, *Aenasius bambawalei* (= *A. arizonensis*) has high searching ability, short life cycle and high parasitism rate (upto 93%). One of the pre-requisites to achieve successful biocontrol program is the availability and survival of the biocontrol agents before the pest population emerges.

The current study was designed to establish a baseline for the future biological control programs in Pakistan by monitoring *P. solenopsis* and their associated natural enemies in southern Punjab (Multan). For strengthening the study goals, laboratory rearing techniques of native *A. bambawalei* (= *A. arizonensis*), with view point of their on-farm conservation was also established.

MATERIALS AND METHODS

The study was conducted at farmer fields at five different locations (Basti Malook, Basti Laar, Billiwala, Makhdoom Rasheed and Sariwala) in Multan district. The crop at each location was sown and all the appropriate agronomic practices were duly performed. The cotton mealybug and respective natural enemies population (predators and parasitoids) was recorded to know the trend on cotton crop at Multan district.

Methodology:

Population dynamics of CMB: Five different locations were randomly selected in such a manner that each field was five to ten kilometers apart from each other. The cotton variety FH-142 was sown in the 3rd week of April during both study years (2012 & 2013). The population data for mealybug and its predators and parasitoids was taken at fifteen days intervals. The data were recorded for two consecutive years (2012 and 2013) from cotton. For recording the population data, five inches terminal twigs of 15 cm length were selected randomly from cotton. These samples were brought to the laboratory to record the mealybug population and its natural enemies which were kept for two weeks in the plastic jars (10 kg) covered with the muslin cloth. Population data of predators and parasitoids from collected samples were recorded on daily basis. Mealybug predators and parasitoids, after recording the data, were removed from samples. The parasitoids population was calculated in percentage on the basis of number of mummified mealybugs in each sample.

Rearing of *A. bambawalei:* Population of cotton mealybug was collected from field and reared on potato sprouts under lab conditions. The uniform aged 3rd instar (260) CMB were separated and placed with newly emerged *A. bambawalei* (= *A. arizonensis*) pair in 5 Kg plastic jar having muslin cloth on its lid with six replications. The experiment was conducted in environment growth chamber having five temperatures regimes (20, 24, 28, 32, 36 ºC) and four relative humidity levels (50, 60, 70, 80 %). Each replication comprised of four jars of each treatment. The honey solution was provided on daily basis to improve the performance. The data was recorded after seven, fourteen and twenty one DAR by counting mummified and emerged parasitoids.

Techniques for *A. bambawalei* releases: Three techniques (cards with mummies, adults and mummies with host) were used to release the *A. bambawalei* (= *A. arizonensis*) under field conditions. Cards with mummies were organized and taken from the Biological Control Laboratory, CABI, Multan. Each card comprised of 4 inches white paper having 200 mummies pasted on it. The reared adults from laboratory of uniform aged were released in the field. The mummies with host were shifted to the field. Thirty-five hundred (3500) mummies /adults/acre were released under field conditions. The data were recorded on the basis of mummified mealybugs after seven, fourteen and twenty one DAR.

***Cryptolaemus montrouzieri* Rearing:** Adults of *C. montrouzieri* were collected from CABI. Thirty (30) adults were placed in plastic jars and then released in the transparent celluloid made cage (48 x 17 x 14 cm) having sleeves as well as mesh windows. The net improved aeration to avoid the growth of sooty mold. Mealybug infested pumpkin were used for oviposition until they were perished. The adults prey all stages of cotton mealybug. The grubs began to appear after one week of egg laying, while the pupae after three and half week, and the adults after approximately forty days (*Sidhapara etal*.,2013). Each cage was put under different ecological conditions (20, 24, 28, 32, 36 ºC temp. and 50, 60, 70, 80 % RH) having five replications.

The green cloth pieces along with folding papers were placed in the cages. The grubs of *C. montrouzieri* were examined in the rearing cages. Then, grubs were shifted on potato sprouts having cotton mealybug. The counting was done and potato sprouts with *C. montrouzieri* were then shifted to the jars, maintained for the pupation. These grubs were also separated from cages and after counting put into the petri dishes and mealybugs were given as food in each petri dish. The observations were made for pupation. It was also observed that the cannibalism was present in *C. montrouzieri*. To overcome this cannibalism a plastic sheet apparatus (1×1 feet) has been designed having 144 blocks of one square inch. A wire gauze was fitted as ventilation source, underside of this apparatus and a moving lid was prepared at the top to avoid the grub's movement to another block. Data were collected to find most suitable conditions for rearing.

Techniques for releases: Two different techniques were used in releases of *C. montrouzieri*, because both larvae and adults are predaceous. In this respect, one thousand (1000) adults / larvae were released (Sarwar, 2016) in an acre in the cotton mealybug infested field and the data were collected after one, three and seven DAR.

Natural enemy field reservoir (NEFR) for the management of CMB: For on-farm mass multiplication of *C. montrouzieri* and *A. bambawalei* (= *A. arizonensis*), natural enemy field reservoir (NEFR) was established and further tested with field pretreatment population. For this purpose, a shed (15×10×12 feet) was constructed in the center of 5-acre cotton experimental area. Under the shed, eight field mass iron rearing trays (2×4×2 feet) were placed. In order to avoid mealybug escape from trays, grease bands were wrapped along these mass rearing trays and their legs were submerged in water filled bowls. A water channel of 9 inches width was constructed around the shed and filled with kerosene mixed water to prevent the entry of mealybug in the field. In each tray, mealybug infested leaves, twigs, branches, fruiting parts of host plant were placed to rear mealybug natural enemies which were replaced on regular basis. The assistance / small releases of both natural enemies were given at NEFR to support the population level. The preferred hosts were grown near NEFR to conserve natural enemies.

Three mealybug infested fields (100, 200 and 400 meters) away from the shed were selected and the data of natural enemies *C. montrouzieri* and *A. bambawalei* (= *A. arizonensis*) and mealybug were recorded before construction of shed (pretreatment data). After the construction, provision and application of all material, the data were recorded for natural enemies *C. montrouzieri* and *A. bambawalei* (= *A. arizonensis*) and mummified mealybug after seven, fifteen and twenty two days from nine (9) randomly selected plants. A set of nine plants was considered as a replication. The cost benefit ratio (CBR) of NEFR technology was also calculated (Bajwa *et al*., 2018).

Statistical Analysis: The data were analyzed statistically using Statistix 8.1 (Analytical software, 2005). Factorial ANOVA was done and means were compared following LSD test.

RESULTS

Fig. 1 presents the overall population trend of cotton mealybug (CMB) during 2012 and 2013. The results clearly revealed that CMB population per twig was recorded 41.35 and 70.70, at the end of May which was statistically different at fortnight interval. Population increased gradually and steadily while reaching at first peak on 25th July (331.75 and 347.25 per twig, during 2012 and 2013 respectively). The highest CMB peak reached on 10th August (381.25 and 422.35, during 2012 and 2013 respectively). Then per twig population of CMB started decreasing onward from 25th August (281.6 and 367.45) and minimum population was recorded on 25th September (28.6 and 30.40 during 2012 and 2013).

During the survey, six natural enemies including five predator species and one parasitoid were recorded predating / parasitizing the CMB population, during the both study years (table 1). The data also described that all the natural enemies remained present throughout the study period.

Fig. 2 reveals that 70% relative humidity with 28 °C temperature was found the most suitable and exhibited maximum *A. bambawalei* (= *A. arizonensis*) population. The highest parasitism on CMB, 64.83, 132.867 and 168.34 followed by 47.75 and 41.42, 68.68 and 51.276 and, 96.932 and 61.25 at 70% RH and 32 °C temperature, and 70% RH and 24 °C temperature, after seven, fourteen and twenty-one DAR, respectively. While minimum population and parasitism was recorded in condition with 50% RH and 20 °C temperature. The rearing of life stages of *C. montrouzieri* under four levels of RH and five levels of temperature revealed that 70% RH and 28 °C temperature was the optimum and exhibited duration, i.e., 2.72, 2.49, 1.831, 60.3 and 75.2 days to complete 1st, 2nd, 3rd instars and adult its life stage whereas 2.35 days taken by 4th instar grub at 70% RH & 24 °C temperature (Figure 3).

Table 1: Mean Trend of predators and parasitoid population associated with CMB during 2012 and 2013.

	Brumus suturalis	***Scymnus coccivora***	***Menochilus sexmaculatus***	***Hyperaspis maindroni***	***Chrysoperla carnea***	***A.bambawalei* parasitism**
25 May	2.250 c	3.250 d	5.750 a	6.750 a	0.750 c	38.563 a
10 Jun	5.750 a	5.250 ab	6.250 a	1.250 bc	1.250 bc	20.695 b
25 Jun	5.500 a	5.000 abc	6.250 a	1.000 bc	1.000 c	20.195 b
10 Jul	6.500 a	6.500 a	4.500 b	2.250 b	2.750 a	11.805 c
25 Jul	6.250 a	6.250 a	4.250 b	2.000 b	2.500 ab	16.108 bc
10 Aug	3.750 b	4.250 bcd	3.750 b	1.250 bc	1.250 bc	16.527 bc
25 Aug	3.500 bc	3.750 bcd	3.500 b	1.000 bc	1.000 c	11.438 c
10 Sept	3.250 bc	3.500 cd	1.250 c	0.250 c	1.750 abc	43.708 a
25 Sept	3.000 bc	3.250 d	1.000 c	0	1.500 abc	42.807 a
LSD value	1.368	1.623	1.001	1.318	1.298	7.344

*Means showing same letter are similar with each other.

The most suited conditions for fecundity are 70% RH and 28 °C temperature recorded to produce maximum eggs, i.e., 238.43 eggs in a whole life. Whereas minimum number of eggs and maximum duration at 50% RH and 36 °C temperature.

The figure 4 showed that 407.04, 409.266 and 406.4 mean number of mealybugs were recorded before the releases of *A. bambawalei* (= *A. arizonensis*) in three selected fields. The rate of percent parasitism was recorded 40.31, 60.6 and 73.998, 32.16, 44.47 and 56.62 and 27.74, 39.33 and 49.91 after seven, fourteen and twenty one DAR, in case of mummies with host, cards and adults released fields, respectively.

Effective releases for larvae and adults of *C. montrouzieri* are presented in Table 4. The results showed that 402.92 and 403.89 mealybugs were examined before adult and larval release. The results indicated population decrease, i.e., 315.716 and 338.78 mealybugs after one day followed by 162.983 and 237.96, and 97.856 and 166.37 mealybugs after three and seven days, respectively. The results also indicated that *C. montrouzieri* adult population was more than larvae after one, three and seven days, respectively (Figure 5).

The results (Table 2) depicted that all the treatments differed significantly from each other. The data also showed the CMB population per twig was statistically similar with other, i.e., 536.67, 537.19 and 537.56 at 100, 200 and 400 meters, distance respectively on pre-treatment. This population per twig started to decrease slowly but steadily by leaving CMB population 294.72, 362.17 and 404.3, followed by 167.58, 251.11 and 297.64, and 32.11, 94.83 and 121.83 individuals after seven, fifteen and twenty-two days at 100, 200 and 400 meter distance, respectively.

In case of *A. bambawalei* (= *A. arizonensis*) the parasitism was statistically similar with other, i.e., 2.22, 2.083 and 1.83 at 100, 200 and 400 meters, distance respectively, on pre-treatment observation. This percent parasitism started to build-up slowly but steadily 19.528, 13.444 and 8.333 followed by 42.639, 33.25 and 24.139, and 56.694, 48.75 and 33.611 after seven, fifteen and twenty-two days at 100, 200 and 400 meter distance, respectively.

The results for *C. montrouzieri* predation was statistically similar with other, i.e., 0 at 100 and 200 meters, distance respectively, on pre-treatment observation. This predation started to build-up slowly 1.639 and 0.861 followed by 3.528 and 2.694, and 4.305 and 3.889 after seven, fifteen and twenty-two days at 100 and 200 meter distance, respectively.

Cost Benefit Ratio of NEFR Technology: The cost benefit ratio (CBR) reflected that with the expenditure of one rupee (01) on NEFR technology, the farmer got benefitted at the rate of 1.81 and 3.257 when the crop was damaged 20 to 50 percent by CMB (table 3).

Table 2. Trend of CMB, *A. bambawalei* and *C. montrouzieri* population at and nearby NEFR.

	Distance (meter)	Population of CMB	Population of *A. bambawalei*	Population of *C. montrouzieri*
Pre-treatment data	100	536.37a	**2.22 g**	0
	200	537.19a	2.083 g	0
	400	537.56a	1.83 g	-
After 7 days	100	294.72d	19.528 e	1.639cd
	200	362.17c	13.444 f	0.861d
	400	404.03b	8.333 f	-
After 15 days	100	167.58f	42.639 c	3.528ab
	200	251.11e	33.25 d	2.694bc
	400	297.64d	24.139 e	-
After 22 days	100	32.11 i	56.694 a	4.305a
	200	94.83 h	48.75 b	3.889ab
	400	121.83 g	33.611 d	-

*Means showing same letter are similar with each other

Table 3: Cost benefit ratio of NEFR technology (01USD = 155 Rs.)

Cost of Mud Shed (15×10×12 feet) = Rs. 200,000/- (Useable for 10 years)	
Cost of Iron Trays	= Rs. 12000/- @ 1500/ tray (Permanent)
Labour Charges	= Rs. 1000/- (Permanent)
Cost of Grease	= Rs. 5000/-
Cost of Kerosene Oil	= Rs. 5000/-
Others	= Rs. 4000/-
Total Expenditure	= Rs. 35300/-
Average Production per Acre	= 25 maunds
Value per Acre	= 25 × 2300 = Rs. 57500/-
Crop damage ranged between 20 to 50%	
So	
Crop damage averted due to NEFR = 20% of net produce, i.e., 5 maunds	
Net Saving	= 5 × 4 acre = 20 maunds
Value	= 20 × 2300 = Rs. 64000/-
Cost Benefit Ratio (CBR)	**= 1: 1.813**
Crop damage averted due to NEFR = 50% of net produce, i.e., 12.5 maunds/acre	
Net Saving	= 12.5 × 4 acre = 50 maunds
Value	= 50 × 2300 = Rs. 1,15,000/-
Cost Benefit Ratio (CBR)	**= 1: 3.257**

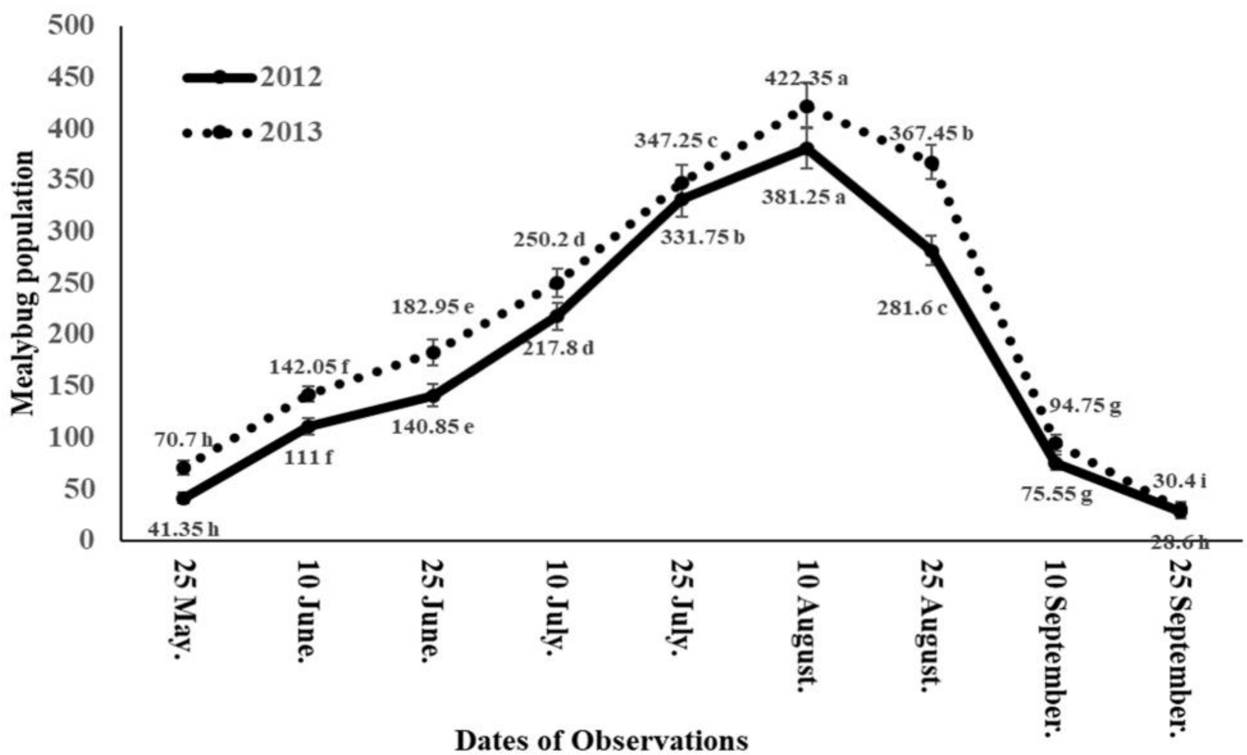

Fig. 1. Trend of CMB population during both study years (2012 and 2013).

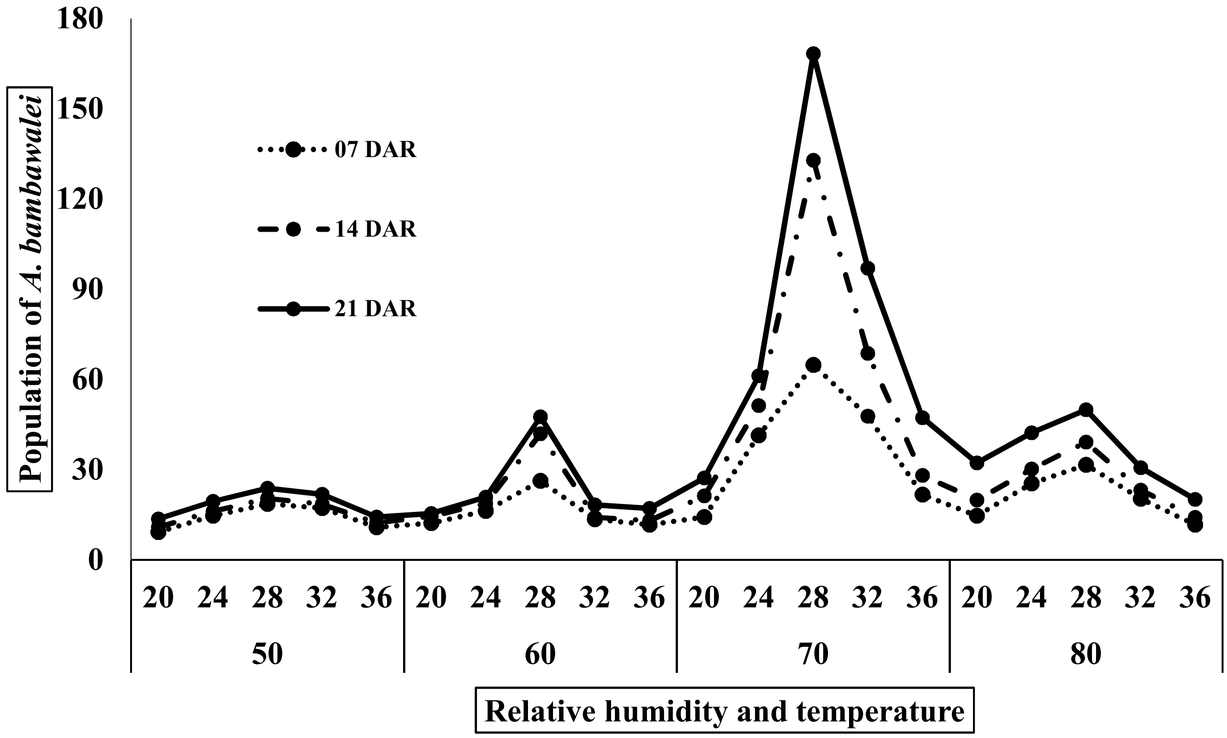

Fig. 2. Rearing of *A. bambawalei* under laboratory conditions.

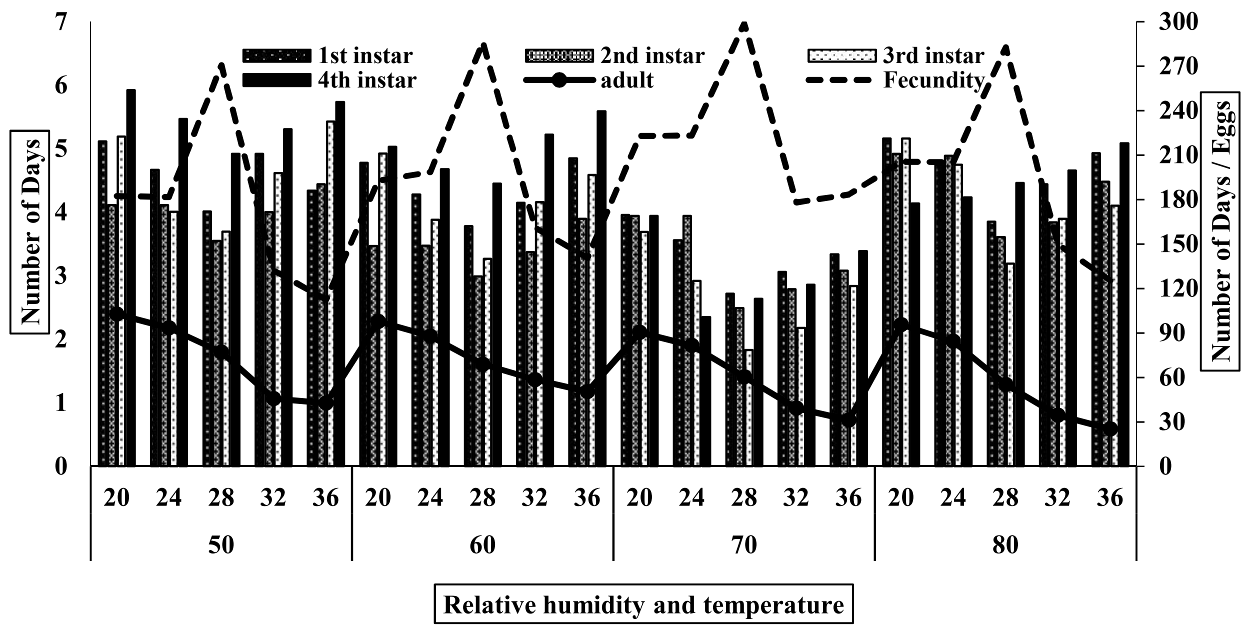

Fig. 3. Rearing technique of *C. montrouzieri.*

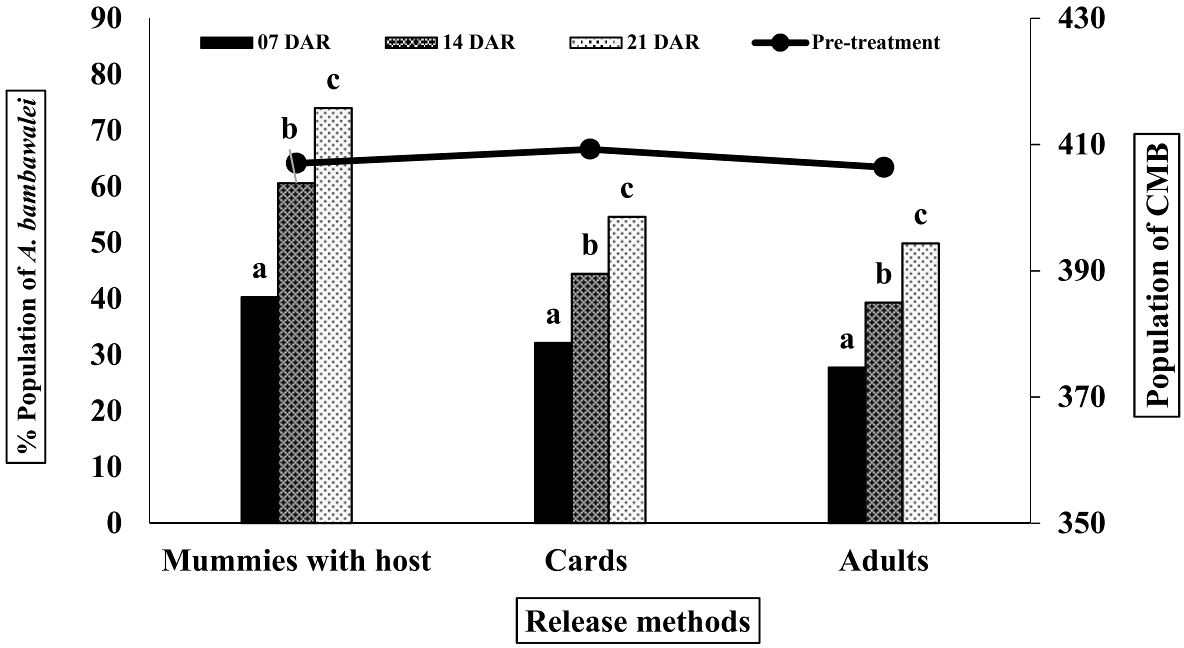

Fig. 4. Release techniques of *A. bambawalei.*

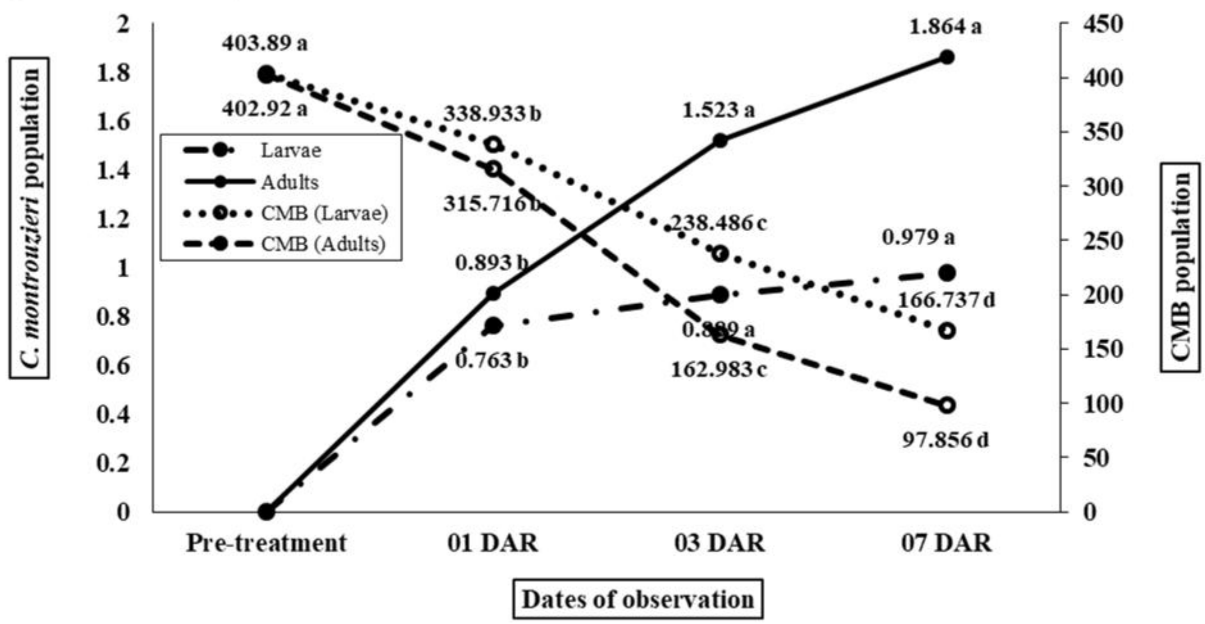

Fig. 5. Release techniques of *C. montrouzieri* On-farm conservation of *A. bambawalei* and *C. montrouzieri* for management of *P. solenopsis*

DISCUSSION

The field surveys showed similar trend at all the locations (Basti Malook, Basti Laar, Billiwala, Makhdoom Rasheed and Sariwala), of Multan district. However, the peak population per twig was recorded 381.25 and 422.35, on 10th of August, during 2012 and 2013, respectively. It is further added that CMB remained prevalent on the cotton crop from May to end of September and the population pressure and intensity increased in 2013 study. This is because of regular evidence on the CMB under the field. Moreover, land was never fallow and each time cotton was sown in the same field so intensity of the pest multiplied. Six different natural enemies were found predating cotton mealybug. These natural enemies consisted of five predators and one parasitoid. These results are in agreement with Sreedevi *et al*. (2013) and Noureen *et al*. (2016) who recorded prevalence and destruction of cotton mealybug during Kharif season and then shifted to other plant species. The most favourable micro and macro climatic conditions such as temperature, relative humidity and wind speed encouraging the cotton mealybug population prevailed during the month of August.

Field prevalence and parasitism of *A. bambawalei* (= *A. arizonensis*) was observed by Ram *et al*. (2009) against cotton mealybug at Haryana, India. In Pakistan, Arif *et al*. (2012a and 2012b) surveyed cotton crop and recorded parasitoid, *A. bambawalei* (= *A. arizonensis*) and predators, *Brumoides suturalis*, *Scymnus coccivora*, *Menochilus sexmaculatus* and spiders. Cotton mealybug mummies (per 6 inch-twig) were observed on shoe flower followed by cotton, tomato, okra, sunflower, silvery and brinjal. Highest parasitism was observed 81.3% on shoe flower followed by cotton, tomato, sunflower, okra, silvery and brinjal. All these results are in line with ours.

Earlier researchers (Zain-ul-Abdin *et al*., 2013, Iqbal *et al*., 2016 and Zhang *et al*., 2016) worked in-vitro, comprehensively on the growth and biological characteristics of *Aenasius bambawalei* (= *A. arizonensis*) on rate of parasitism with respect to synchronization. Three different techniques (adults, mummies with cards and mummies with host) were used for *A. bambawalei* (= *A. arizonensis*) releases but mummies with host proved the most effective and efficient method than cards and adults.

The results showed that the CMB pre-treatment population per twig was 536.67, 537.19 and 537.56 at 100, 200 and 400 meters, distance. This population started to decrease slowly but steadily in response to build-up of natural enemy by reducing CMB population 294.72, 362.17 and 404.3, followed by 167.58, 251.11 and 297.64, and 32.11, 94.83 and 121.83 individuals after seven, fifteen and twenty-two days at 100, 200 and 400 meters distance, respectively. In the same pattern, the *A. bambawalei* (= *A. arizonensis*) population was also built-up in terms of number and distance (dispersal). The results showed that the *A. bambawalei* (= *A. arizonensis*) parasitism before reservoir construction was 2.22, 2.083 and 1.83 at 100, 200 and 400 meters, distance respectively. This percent parasitism started to increase steadily 19.528, 13.444 and 8.333 followed by 42.639, 33.25 and 24.139, and 56.694, 48.75 and 33.611 after seven, fifteen and twenty-two days at 100, 200 and 400 meter distance, respectively. Whereas the increasing trend observed in case of *C. montrouzieri* 1.639 and 0.861 followed by 3.528 and 2.694, and 4.305 and 3.889 after seven, fifteen and twenty-two days at 100 and 200 meter distance, respectively.

Classical biological control is control of insect with its native Bio-control agents whereas inundative means large releases of biological control agents to supplement the population in a definite period of time. Earlier investigations (Rosas-Garcia *et al*., 2009; Atif *et al*., 2011) evaluated different life stages of *C. montrouzieri* against citrus mealybug and concluded efficiency of all stages. But earlier instar had less demand to consume the administered feed, so these 1st and 2nd instars consumed lesser amounts of mealybugs whereas later developmental stages such as 3rd instar and adults showed more thrust of food. These stages devour the mealybugs in less time and consume higher number of mealybugs (Ahi *et al*., 2015) and hatching percentage is also increased remarkably. This study will not only provide the model for future *P. solenopsis* IPM programs but also will lead scientists towards its classical biological control in Pakistan.

Conclusions: Natural enemies especially *A. bambawalei* (= *A. arizonensis*) and *C. montrouzieri* can effectively manage the building population of CMB when they are conserved properly through establishment of natural enemy field reservoir (NEFR) and can reduce the crop losses.

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