Comparative Crop Production and Protection Costs of Cotton Crop under Different Pest Management Strategies of Cotton Mealybug, *Phenacoccus Solenopsis* (Tinsley)

Hakim Ali Sahito^{1*}, Tasneem Kousar¹, Wali Muhammad Mangrio¹, Bhugro Mal², Abdul Hafeez Mastoi³, Yasir Akbar Jamali⁴, Faheem Ahmed Jatoi¹ and Azmat Hussain Abro⁵

¹Department of Zoology, Faculty of Natural Sciences, Shah Abdul Latif University Khairpur Mir's 66111Sindh-Pakistan.

²PARC-Southern Zone Agricultural Research Centre, Karachi, 75270 Sindh, Pakistan. ³Department of Entomology, LUAWMS, 90150 Uthal, Balochistan

⁴Institute of Microbiology, Faculty of Natural Sciences, Shah Abdul Latif University Khairpur Mir's 66111 Sindh-Pakistan.

⁵Department of Geology, University of Sindh, Jamshoro, 71000, Sindh-Pakistan.

Corresponding author: Email: hakim.sahito@salu.edu.pk; Ph. #. 00 + (92) 301-3515723.

Abstract

The research work was conducted to understand the comparative crop production, protection costs, and parasitization efficiency of Aenasius bambawalei on Phenacoccus solenopsis under cotton cultivated crop. When 2nd instar mealybugs were offered for parasitization, the sex ratio was male-biased under laboratory conditions. The parasitization rate was observed high on adult mealybugs compared with the 3^{rd} instar and no parasitization was observed on the 3^{rd} instar of mealybugs. Parasitization of mealybug was highest on cotton (36.34%) and the lowest on Trianthema portulacastrum (13.98%) compared with other host plants. The applying insecticides and biological control modules using the natural enemy's field reservoir (NEFR) were observed on cotton crop, 2021. Mealybug parasitization was found highest on cotton (36.34%) and lowest on horse purslane (13.98%) compared with other host plants. The seasonal mean population of P. solenopsis, parasitized pest mummies, and predators were 27.40±0.6, and 19.10±0.20 per 10cm twig per plant, and 5.46±0.30 per plant, respectively in NEFR cotton. The mean population of A. bambawalei recorded on farmer's cotton was 8.35±1.62, and in NEFR 15.75±1.54 parasitized mealybug mummies per 10 cm twig per plant. The Promuscidae unfasciativentris, hyperparasitoids population was 1.59 per plant twig with 18.38% parasitization of A. bambawalei on farmer's field compared with 3.39 and 17.17 % on NEFR cotton crop. Economic analysis of different pest management strategies showed that the chemical control strategy of farmers gave more than double income compared to NEFR.

Keywords: Hyperparasitoid, Pest, Predators, Promuscidea unfasciativentris.

INTRODUCTION

The *Phenacoccus solenopsis* Tinsley is a widely distributed, exotic species and polyphagous in feeding behavior severely damaging more than 146 plant varieties (Hodgson et al., 2008). This species of potential pest insects are frequently found in ornamentals, weeds, horticultural crops, and medicinal plants (Kousar et al., 2016). The cotton mealybug damaged leaves, branches, main stems, fruits, trunks, flowers, foliages as well roots that feed on phloem (Sahito et al., 2012). The

cotton mealybug outbreak on cotton varieties and other plants was first reported in 2005, and 206-7 more than 12% cotton crop was lost at 2.4 million bales, even infestation in Pakistan increased, 2008 (Afzal et al., 2009). In India, from different cotton varieties, the P. solenopsis were reported which caused gradually economic loss (Nagrare et al., 2009). The P. solenopsis is considered as the high-risk invasive international pest species globally causing significant threat to horticultural crops in subtropical and tropical regions of the world (Wang et al., 2010). Throughout the world, citrus and several other valuable plant families are damaged by economic pest insects through their larval stages (Mangrio & Sahito, 2022), usually, these stages of the pest insect species serve as destructive agents to their host plant communities (Mangrio & Sahito, 2021). On the functional response of A. bambawalei the effect of the chlorpyrifos and spirotetramant was studied (Hadian et al., 2020). Moreover, on demographic traits of A. bambawalei the sub-lethal effects of the thiodicarb, imidacloprid and dimethoate were investigated (Rafatian et al., 2021). The P. solenopsis is the sap-sucking and soft-bodied pest insect distributed throughout the world, they are phloem feeders and are so-called mealybug because of them around their bodies the mealy wax secretion (Subramanian et al., 2021). The cotton mealybug causes considerable economic losses in Pakistan, India, China and the primary control practice over this pest insect is insecticidal application but for the management of the nymph stages biological control is a strong potential remedy (Waqas et al., 2021). The differences in the type, composition of the selected insecticides, experimental condition, and natural enemy sensitivity level may the reason for the variation in the results (Asadi et al., 2019). During the research work, attention was given to P. solenopsis host plant preference and its effect on A. bambawalei parasitization, host life stage preference under field examinations. The natural enemy's role in P. solenopsis population regulation and their biological controlling agents were evaluated under field conditions. The success impact of hyperparasitoid, A. bambawalei for effectual biological control of P. solenopsis and population management approaches were evaluated.

MATERIAL AND METHODS

The *P. solenopsis* and *A. bambawalei* population fluctuation on cotton and other host plant varieties at the Sharif Model Farm, District; Naushahro Feroze, Sindh the cotton crop (cv.NIAB-78) was sown on 5th May, 2021 and the different other alternate host plants such as; China rose, Okra, Horse purslane, Abutilon, and Brinjal were cultivated surrounding the ridges. Through weekly intervals, the cotton mealybug population was recorded randomly from (25) plants. Frequently stems, flowers, new emerging leaflets, leaves buds of the cotton plants were examined and for further observation, the selected plants were tagged with red cotton ribbon. At 10 cm twig/plant the adult females with visible 3^{rd} instar of the mealybug with associated parasitized mealybug mummies on all host plants were counted. The instars population of the *P. solenopsis* was collected from the field conditions and for parasitoids emergence kept in Petri dishes to ascertain the population of the *A. bambawalei* for parasitization of suitable host range selection and six times experiment was replicated.

The P. solenopsis population management through different strategies

To investigate the comparative efficacy of mealybug parasitiod at insecticides farmers' field and natural enemy's field reservoir (NEFR) field investigation was conducted. Ten-acre field was reserved at a distance of two hundred meters for insecticide application and (NEFR) individually, (cv. Bt.21) cotton was cultivated and kept 60 and 22 cm distance between rows and plants. The

different agronomical approaches such as; weed management, application of fertilizers, irrigation, etc., were applied according to their need based on both fields.

Farmer's field

The insecticides were applied at the farmer's field on the schedule and choice of the farmer. The pesticide application was made by the farmer after the presence of the population of mealybug on their judgment. The plants were sprayed with the help of a shoulder-mounted knapsack sprayer and through the calendar spray schedule method farmers followed spraying the crop on the alternate week. After spraying for one week the pest insect data was gathered from farmers' fields at a fortnightly interval basis and for this purpose (50) plants were randomly selected for pest and parasitiod count. At the 10 cm length of infested plant twig on per plant data was recorded. At cotton farmer fields used different insecticides; 1st spray; Diafenthinron 500 EC, dose 200 ml/acre, 2nd spray; Diafenthinron 500 EC + Acephate 75 SP, dose 250 + 150 ml/acre, 3rd spray; Imidacloprid 20 SL + Acetamiprid 20 SP, dose 250 + 150 ml/acre, 4th spray; Dettol + Imidacloprid 50 EC, dose 100 + 250 ml/acre, 5th spray; Imidacloprid 50 EC + Propfenofos 200 SL, dose 150 + 1000 ml/acre and 6th spray, propfenofos 200 SL, dose 1000 ml/acre, respectively.

Natural enemies field reservoir (NEFR)

In the middle of (10) cotton crop on 335×609 land piece with cm in height, the natural enemies' field reservoir was built. The shed pillars were made of bricks with cement and cemented shed floor, the ceiling was made with leaves of date palm and bamboo matrix, underneath of date palm leaves a plastic sheet was made to protect from sunshine and rain. Twenty iron cages of 90x60 cm under the shed were kept as the population of *P. solenopsis* and parasitiod reservoirs. At the upper portion of the iron cage wall, the grease oil was pasted because no mealybug of crawler could leave the cage. The water bowls having a few drops of mobile oil were placed under the cage pods to prevent the insects and predators into the cages. Around the shed, a water channel was made to keep water for twenty-four hours and this was done to escape the crawler's disappearance and movement from the field to the cages. The population of the mealybug was kept in the cages to invite parasitoids and in the cage, the parasitoids had a chance to disperse in the field to parasitize the mealybugs and through this activity, the parasitoids were enhanced.

In the natural enemy's field reservoir for the stock maintaining culture of mealybug, the infested plants containing mealybug kept inside the cages, get pest insects separated and poured in Petri dishes with the camel hair brush source and released in natural enemies field reservoir cages. The bottle ground and potato sprouts were given to the population of the mealybug as a food source. In the laboratory the stock culture of *A. bambawalei* was maintained on the cotton mealybug, after infestation on mealybug on cotton, *A. bambawalei* mass culture was produced and parasitoid was provided *P. solenopsis* from insecticide-free cotton. The 1st released (10,000) parasitoid, *A. bambawalei* was made in the natural enemies field reservoir to parasitize the cotton mealybug in June, subsequently released 2nd replication in July and 3rd replication in August, after one-month intervals. The infestation of the pest and parasitization data were taken after fifteen days intervals and randomly fifty plants were selected for observation to count the population of pest insects and parasitoids at natural enemy's field reservoirs. From infested plants, the data was recorded up to 10 cm in length and per plant, the population of those predators was recorded which were found to feed on the nymph and adult stages of mealybugs.

Occurrence of a hyperparasitoid, Promuscidae unfasciativentris Girault (Hymenoptera: Aphelinidae) on A. bambawalei

During the research studies of the *A. bambawalei*, parasitism rate on mealybug, the first time a new parasitoid was found from mealybug, the *A. bambawalei* in May 2021, and through available literature, the parasitiod specimens were confirmed. A new species of hyperparasitoids, *Promuscidae unfasciativentris* Girault was identified on *A. bambawalei*, respectively. The populations of mealybugs were collected from both natural enemies fields farmer sprayed and un-sprayed and (20) mummified were kept in the vial (7.0 x 3.0 cm). The experiment was replicated ten times under laboratory conditions at $25\pm2^{\circ}$ C throughout the cotton crop season, 2021. The hyperparasitoids data was collected from the beginning up to the harvest of the cotton crop after fifteen days intervals and both population management strategies of mealybugs were analyzed using t-test through a statistical package using SWX, 8.1 statistics software.

RESULTS

The highest parasitization percent of cotton mealybug by *A. bambawalei* was recorded on the cotton crop, compared with Okra and Abutilon under field conditions and the minimum population of parasitization was recorded on Horse purslane (Fig.1). The parasitization rate of the 3^{rd} instar, cotton mealybug was found with a significant difference (T=16.29, DF=5; P<0.001) lower (54.50±5.99) than female adults (72.0±4.03), respectively.

The P. solenopsis and A. bambawalei population fluctuation on cotton and alternate host plants

Under field conditions, the highest percent parasitization of the population of *P. solenopsis* by *A. bambawalei* was found on cotton, followed by Okra and Abutilon, and on Horse purslane less population was recorded (Fig. 1). On the 3rd instar of *P. solenopsis*, the rate of parasitization was lower (54. \pm 5.99, $\overline{x} \pm$ SE) than in female adults (72.0 \pm 4.03), respectively.

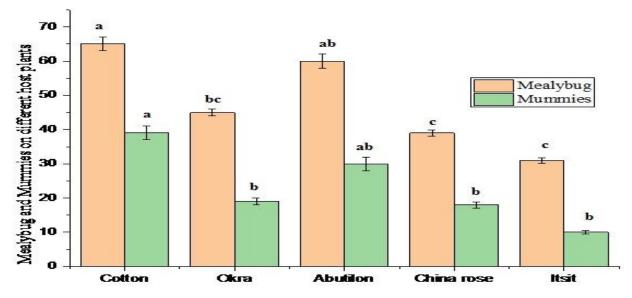


Fig. 1. Mean (±SEM) population of *P. solenopsis* and *A. bambawalei* on cotton and alternate host plants summer season during, 2021. The *P. solenopsis population fluctuation at farmer's field*

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The infested cotton containing mealybug population was recorded from May 2021 with (3.40) pest insect's prevalence on the twigs per plant and the 1st spray was done 5th June 2021. The population fluctuation of the mealybug remained throughout the season even with the application of insecticides at 12.70 on the twigs of the plants and on June 20th, 2021 the minimum number of parasitized pest mummies was found at the starting application of insecticide time. The last application of insecticides was made in the 1st week of September and farmers started cotton picking in August. The activity of parasitoids increased after the reduction of presticide pressure and on October 18, 2021, the parasitization was recorded (14.65%) with overall predators mean population of 1.98 per plant (Fig. 2).

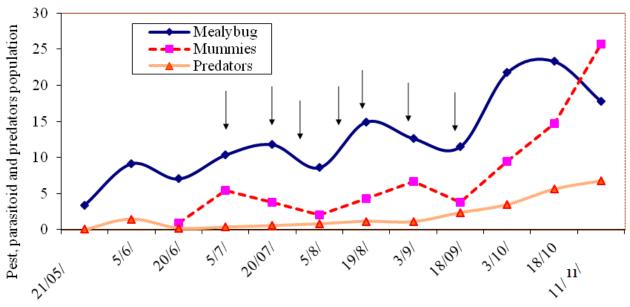


Fig. 2. Mealybug parasitized mummies and predator population in farmer's cotton crop. The vertical arrows show the application of insecticides by the farmer.

The P. solenopsis population fluctuation on the Natural enemy's field reservoir cotton crop

The population of mealybug from May 21, 2021, and the population increased with a high peak at 43.95 in NEFR per twig on August 5, 2021. When in the field condition the activity of the parasitoid increased the population of the mealybug decreased. There was a little number of parasitized mummy's earlier stage of the crop at 0.40 per twig/plant on June 5, 2021. After that, the parasitoid maximum activity of the parasitized pest mummies increased and reached up to 42.25 per twig/plant during, October 3, 2021, and until the crop was harvested the almost same numbers were recorded. The mean seasonal population of mummies was recorded at 19.09 per twig and the predator's mean population was recorded at 05.46 per plant (Fig. 3). The population development and parasitization percent of the pest and predators of *P. solenopsis* found significantly high in NEFR treatment compared with farmers (T= 2.28; DF= 11; P= 0.021) pest, (T= 2.25; DF= 11; P= 0.045) predators and (T= 2.72, DF= 11; P= 0.044) parasitization treatment.

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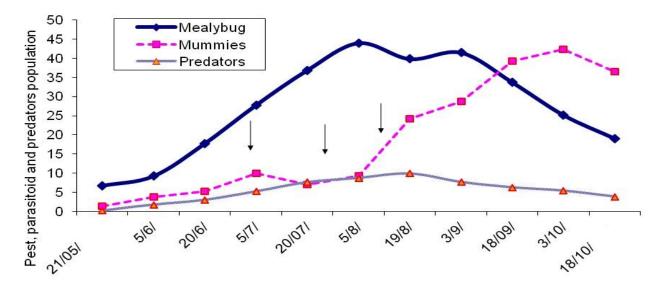


Fig. 3. Mealybug parasitized mummies and predator population in NEFR cotton crop. The vertical arrows show the release of the parasitoid, *A. bambawalei*.

The hyperparasitoids, P. unfasciativentris population on A. bambawalei at NEFR, and farmer's field crop

The mean population of *A. bambawalei*, parasitiod at farmers field was recorded (8.35 ± 1.62) per twig/plant parasitized pest mummies and *P. unfasciativentris*, hyperparasitoids population recorded 1.59, per twig/plant parasitized mummies on per plant at 12.38% of *A. bambawalei* parasitization shown in (Table- 1). The *A. bambawalei* mean population in NEFR parasitized mummies per twig/plant were recorded at 15.75 \pm 1.54, and *P. unfasciativentris*, hyperparasitoids population recorded at 3.39 per twig/plant parasitized with 17.17% parasitization. The *A. bambawalei* parasitized pest mummies in NEFR treatment was significantly higher compared with farmer's field (T= 4.88; DF= 11; P= 0.001). The *P. unfasciativentris*, hyperparasitoid population was also found significantly higher (T= 3.62; DF= 11. P= 0.004) with (T= 1.76; DF= 11; P= 0.106) percent parasitization in NEFR compared with farmer's conton field.

Table- 1. The population of hyperparasite, Promuscidea unfasciativentris on A. bambawalei
at NEFR and farmer's field on cotton crop

Data of		The population of farmer field			Population of NEFR		
Date of observation	Sprays	Parasitized mealybug mummies	P. unfasciat- iventris	P. unfasciat- iventris %	Parasitized mealybug mummies	P. unfasciat- iventris	P. unfasciat- iventris %
21/05/202 1	-	7.60±0.56	0.00±0.00	0.00	15.45±1.37	0.95±0.17	5.79
5/6	-	11.70±3.21	0.35±0.18	2.90	17.30±2.76	1.20±0.56	6.48

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	20/6	1	5.95±1.26	0.05±0.33	0.83	13.65±1.23	0.85±0.13	
	5/7	2	7.35±1.76	0.70±0.98	8.69	19.40±1.87	2.45±0.37	
	20/7	3	4.50±0.67	0.00±0.00	0.00	17.65±1.54	4.15±1.13	
	5/8	4	8.90±2.76	0.95±0.13	9.64	16.30±0.87	3.90±0.96	
	19/8	5	5.35±2.56	1.80±0.56	25.17	11.40±0.57	1.30±1.47	
	3/9	6	5.80±0.97	0.10±0.87	1.69	13.85±1.54	3.85±0.93	
	18/9	7	4.95±0.57	1.40±0.33	22.04	18.10±1.46	4.50±0.98	
	3/10	-	9.20±1.46	2.65±0.39	22.36	17.35±2.13	5.55±1.86	

4.75±1.23

6.35±1.87

 1.59 ± 0.57

Yield and income of cotton crop from the farmer's field and NEFR

13.10±1.93

 $15.85{\pm}1.68$

8.35±1.62

The cotton crop yield and income obtained by adopting different pest management strategies indicated that the chemical control strategy gave farmers more than double income compared with the biological control (NEFR) strategy (Table- 2).

26.61

28.6

12.38

19.60±1.23

 $8.95{\pm}1.87$

 15.75 ± 1.54

Table- 2. Comparative crop production and protection costs of cotton under different pest
management strategies (per acre)

Particulars	NEFR	Farmer
Ploughing	US\$. 187.5	187.5
FYM	US\$. 75	-
Labour charges (weeding, irrigation)	US\$. 131.25	156.25
Fertilizer (DAP + Urea)	US\$. 185	185
Parasitoid rearing + release	US\$. 51.25	-

18/10

7/11/2021

≖±SE

-

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5.86

11.21

19.03

19.30

10.23

21.75

19.91

24.23

25.75

36.52

17.17

 6.80 ± 2.56

 5.15 ± 2.13

 3.39 ± 1.10

Shed for NEFR + maintenance of mealybug culture	US\$. 175.0	-
Insecticides application	-	318.7
Picking charges	US\$. 50.6	78.7
Transport charges	US\$. 62.5	125
Government revenue charges	US\$. 12.5	12.5
Total expenses	US\$. 930.6	1063.7
Yield / production per acre	27 maunds = 1080 kg	1680 kg
Total income (US\$. 1.187 / kg)	US\$. 1282.5	1995.0
Net income / acre	US\$. 351.9	931.3

DISCUSSIONS

On the *P. solenopsis* nymph instar, no *A. bambawalei* parasitization was found under laboratory conditions and the present study suggest that *A. bambawalei* can recognize the 2^{nd} instar hosts as suitable for female progeny and under field conditions does not oviposit in 2^{nd} instar *P. solenopsis* this is similar with the work agreement of (de Jong & vand Alphen, 1989) found similar host stage preference for *Leptomastix dactylopii* parasitizing citrus mealybug, *P. citri*. and (Mangrio et al., 2020) discussed the food and feed consumption of *P. demoleus* larvae on lemon leaves. The many parasitoids of Encyrtidae were found with a positive preference for stages of parasitism and range of host size (Chong & Oetting, 2006).

Further, the result of our study showed that the cotton mealybug infests from the initial stages of the cotton crops this is with the work agreement of (Abbas et al., 2005) they documented that about 14 percent of the cotton crop is damaged by cotton mealybug in Pakistan. In the field experiment against cotton mealybug, the pesticide application applied by cotton growers provided effective control. The mealybug infestation was recorded from May month and the first spray was carried out June 20, 2021, but throughout season three was continued insecticide application. The picking of cotton started in August and in the first week of September, the farmers made the last application. When the farmers applied insecticides to their cotton crop there was a low population of parasitized pest mummies. The activity of the parasitoids increased when farmers stopped insecticide application is with the similar work of (Shafqat et al., 2007), who described that in cotton crop the *P. solenopsis* predominantly depended on justified and wise use of toxic chemicals and emphasized the developmental need for Integrated Pest Management strategy to combat of cotton mealybug.

In NEFR treatment compared with the farmer field significantly high mean population of the mealybug was recorded same findings reported by (Ram et al., 2009), who conducted studies on the biology of solitary nymph endoparasitoids of mealybug parasitoid, *A. bambawalei* caused 37.6 to 47.2% parasitization of *P. solenopsis*, which increased to 72.3%. The *A. bambawalei* female could parasitize 38-163 mealybugs in its life of 11-35 days and 5-90% parasitization

caused by *A. bambawalei* indicating in biological control the great potential for biological exploitation. In the present research study work, the *A. bambawalei* found an effective natural enemy against cotton mealybug as described that in mealybug population parasitiod caused the decline and there was more than 90% parasitism on cotton in the September month. Same findings are documented by (Tanwar et al., 2011) that the mealybug infestation ranges from low (10-20%) to high (40-60%) in 2007-08 but gradually decreased in 2009. The *A. bambawalei* played a pivotal role in the population of pest insect reduction and at many locations on *P. solenopsis* the natural parasitization could reach more than 90%. In the case of cotton mealybug, severe infestation application of pesticide is needed to protect the cotton crop from destructive pest insects. It is due to the high population build-up of pest and biotic potential in a tropical climate and due to these reasons the *A. bambawalei* prefer the third stage of the pest to parasitize (Fand et al., 2011) but at younger nymph stages of the pest, no parasitiod could become effective to parasitize.

The *P. unfasciativentris*, a hyperparasitoid emerged from mummies of the *P. solenopsis* formed by *A. bambawalei* in the last days of 2021 at Tandojam, and from the Natural History London, British Museum the identity of the hyperparasitoids was confirmed. The *P. unfasciativentris* caused a significant reduction in the population of *A. bambawalei* but the range incidence found varied from 1.0 to 26.7% in different months of the year. This species of hyperparasitoids at Tandojam has been reported to cause a severe decline in the population of *A. bambawalei*. The cotton mealybug infestation upsurge and weaken valuable crop plants (Mahmood et al., 2011). The four species of the hyperparasitoids on *A. bambawalei* have been reported from India (Pala & Sina, 2010). From different location in India, the *P. unfasciativentris* have been documented and earlier had been reported and these species of hyperparasitoids relies on the adverse effects on the population reduction of the *A. bambawalei* (Muniappan, 2009). During survey at different locations from the *A. bambawalei*, the population of *P. unfasciativentris* was found with a positive prevalence.

Hyperparasitoids have adverse effects on biological success (Muniappan, 2009). (Holler et al., 1993) carried out research on cereal fields to clarify the relationship between hyperparasitoids of cereal aphids and their primary parasitoids. The bethylidae wasps are found primary lepidopteron and coleopteran species (Perez-Lachaud et al., 2004). The *Cephalonomia hyalinipennis* is considered as the facultative hyperparasitoids of the bethylidae species and these potential trophic interactions disturb the biological control mechanism. Hyperparasitism depends on the extent of systematically searching their primary parasitoids (Banks et al., 2008). The hyperparasitoids under greenhouse experiments, *Asaphes suspensus* found capable in a population reduction of the *Aphidius ervi* (Schooler et al., 2011).

The cotton crop yield and income obtained by adopting different pest management strategies indicated that the chemical control strategy gave farmers more than double income compared with the biological control (NEFR) strategy. The population management of the mealybug technique in NEFR was applied by (Solangi and Mahamood, 2011) but from farmer cotton fields more crop yield was obtained than NEFR (Mahmood et al., 2011). In the IPM program, the conservation of natural enemies is one of the goals to achieve high predators and parasitiod levels. Natural enemies' conservation can be accomplished through the wise application of

insecticides because an insecticide generally hits the population of the beneficial insects (Reddy, 2011).

The overall results of our findings have closely resembled others studies that *P. solenopsis* is an invasive pest and it is difficult to manage within a single strategy because of its high reproductive potential. The *A. bambawalei* is recognized as the most effective parasitiod of cotton mealybug because in a cotton field other predators could not bring the population of the pest under control alone. For *P. solenopsis* population management in conjunction with pesticides reduce the risk of natural enemies. The imidacloprid WP and neem oil formulation were documented less toxic against the population of the predators and parasitoids, *A. bambawalei* of cotton mealybug compared with other pesticides (Sahito et al., 2011). The neembased insecticide is regarded as an ideal insecticide in IPM inclusion due to less toxicity on the population of natural enemies (Reddy, 2011). Compared with farmer's cotton field significantly high population of the pest, parasitiod, and predators found in NEFR treatment and less parasitization of *P. solenopsis* and fewer incidences of hyperparasitism by hyperparasitoid, *Promuscidea unfasciativentris* on *A. bambawalei* were recorded compared with NEFR.

CONCLUSION

The field and laboratory-based research study was conducted on cotton (cv.NIAB-78) and other alternate host plants to understand the population fluctuation of the *A. bambawalei* on *P. solenopsis* at Sharif Model Farm, Naushahro Feroze, and Entomology Laboratory, Department of Zoology, Shah Abdul Latif University, Khairpur-Sindh, 2021. The mealybug parasitization was found high in cotton and low in *T. portulacastrum*. Under laboratory conditions, *P. solenopsis* were offered for parasitization and found a high prevalence parasitic rate in adults compared with the 3^{rd} instar of *P. solenopsis*. The overall parasitized pest mummies, predator population were also observed per twig/plant in cotton NEFR. The hyperparasitoids, *P. unfasciativentris* population was also seen in NEFR and farmers' cotton crop. For keeping up growing cotton crop production and beneficial economic impact proper management regarding cotton mealybug is abruptly needed in Pakistan. The obtained results are important in the management of *P. solenopsis*. However, more insight is needed for the proper perception to combat this potential pest, the cotton mealybug.

AUTHORS CONTRIBUTION

H.A.S, is the main author of this research article, collected the data from farmers cotton field and NEFR, T.K, carried out the laboratory work, W.M.M, wrote the paper, statistically analyzing the data, B.M, helped in data collection, A.H. M, helped in proofreading, F.A.J, helped in the examination and identification of the hyperparasitoids, Y.A.J and A.H. A, arranged the tools and required materials in the entomology laboratory.

IMPACT STATEMENT

Cotton is the most important cash crop of Sindh, Pakistan. It is the main exportable commodity economic good and exchange earning of the country. But cotton mealybug is a serious pest in cotton-growing areas of Sindh. The wise use of pesticides and attention to enhance the population of biological controlling agents reduce the population of this destructive pest insect species. Therefore, the result of our study may help in this perspective.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AVAILABILITY OF DATA AND MATERIALS

At the request of the corresponding author, the findings and data of this research work are available due to ethical privacy and restrictions.

CONSENT FOR PUBLICATION

For releasing of this research-based material the authors accept all responsibilities

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