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BIOLOGICAL CONTROL AND ANTAGONISTIC ACTIVITY OF NATURAL ENEMIES AGAINST, *PAPILIO DEMOLEUS* AT SAHATI REGION OF SINDH - PAKISTAN

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ABSTRACT

The swallowtail butterfly is the widespread vigorous destructive pest of new growing green leaves of *Citrus limon,* the different species of predators and parasitoids on major pests were observed field and laboratory conditions during, 2022. The overall monthly mean population of lemon butterfly (LBF) was recorded (26.23±5.12) on *C. limon* leaves and the natural enemies were also observed namely; *Peucetia viridans* (0.12±0.34) consequent to *Cheiracanthium punctorium* (0.12±0.34), *Halyomorpha halys* (0.12±0.34), *Agelenopsis* sp. (0.12±0.34), *Delta pyriforme* (0.13±0.36), *Geocoris punctipes* (0.14±0.37), *Aculepeira ceropegia* (0.10±0.31), *Menochilus sexmaculatus* (0.12±0.34), *Coccinella septempunctata* (0.17±0.40), *Brumus* sp., (0.12±0.34), and ectoparasitiods; *Tamarixia radiata* (0.28±0.52), *Tetrastichus* sp., (0.30±0.54). The overall parasitiods population of eggs (11.44±2.00), larvae (6.98±1.22), and pupae (4.27±0.80), hence; the egg parasitoids; *Telenomus* sp., (0.11±0.33), and *Ooencyrtus papilions* (0.10±0.32), the larvae parasitoids, *Distatrix papilionis* (0.13±0.36), *Apanteles papilions* (0.09±0.30), and pupae parasitoids, *Pteromalus puparum* (0.10±0.32) were reported and found with differences in population occurrences. Thus, it is further recommended to keep toxic insecticides free lemon orchards and enhance the activity of natural enemies to reduce the pest population under field conditions.

Keywords: *A. papilions, C. limon, D. papilionis, O. papilions, P. puparum*

INTRODUCTION[1]

Citrus, the prime genus contains thorny aromatic orchards with long domestication history and originated first in the Indian Sub-continent (Rao *et al*., 2021). Citrus is the most significant crop with high content of vitamin C, antioxidant values, and these evergreen plants contain stems with sharp spines with leathery leaves (Gorinstein *et al*., 2021). Brazil is the top most citrus-producing country but nowadays, citrus fruits are being grown in more than 52 countries with healthy production (Mahato *et al*., 2019). In Pakistan this horticultural crop holding as the leading fruit with the highest export share (Burhan *et al*., 2018). The *C. limon* orchards grow in open habitats, seedy or seedless, oblong to obovate, delightful taste, spread upright, therapeutic, widely preferred as health benefits (Czech *et al*., 2021), and are more popular due to their refreshing fragrance, nutritional values, and thirst-quenching quality (Singh *et al*., 2019).

Lepidoptera is the well-known and second-largest order of insects in the world including moths and butterflies (Khan *et al*., 2022). The larvae of the LBF vigorously damaging to the *C. limon* orchards especially new emerging soft green leaves (Mangrio *et al*., 2020). The *P. demoleus* (Lepidoptera: Papilionidae) are widely dispersed insects their larvae pass five distinct stages (Islam *et al*., 2019). These insects behave as habitual potential insect pest species found throughout the year on the new and soft emerging lush green leaves of the citrus varieties (Mangrio and Sahito 2023). The fertilized adult female of *P. demoleus* lays eggs continuously for up to two to three days on leaflets and tender twigs causing massively defoliation (Mangrio and Sahito, 2002). Papilionidae, Eriophyidae, Crambidae, Drosophilidae, Cossidae, Curculionidae, and Pseudococcidae families of insects are major pest species to citrus (Mal *et al*., 2014).

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Several species of butterflies are being targeted by a variety of predators, parasites, and parasitoids (Kunte, 2006). These species of predators and parasitoids are playing pivotal role in decreasing the pest population, especially LBF (Lewis, 2009). Inside the larvae of LBF, the parasitized wasps lay dozens of eggs and feed several vital organs (Singh and Singh, 1998). The adult species of LBF are attacked by Praying mantis, Yellow wasp, Spiders, pupae parasitized by Pteromalus (Atwal, 1964). From the *Danaus plexippus* butterfly, the *Lespesia archippivora* parasitiod was documented (Bartel *et al*., 2011), and *T*. *puparium* from the *C*. *limon* species (Sureshan, 2013). From the larvae of the *Danaus plexippus* butterfly, the parasitiod *Lespesia archippivora* was reported (Oberhauser *et al*., 2007). From the host Skipper butterfly, *P*. *puparium* and *Sympiesis notate* were reported (Sureshan, 2013). *Elasmus*, *Brachymeria*, and *Catolaccus* are the primary parasitoids of hesperiid host species (Richard *et al*., 2022). However, instead of chemical control biological control is a good alternative. Thus; keeping in above mentioned problems created by this vigorous pest, the biological control and their proper identification was kept under observation and highlighted for future research planning from this Sahati region of Sindh-Pakistan.

MATERIALS AND METHODS

Source of host

The present research work was performed to find out the biological control through predators and parasitoids of the LBF at different stages during, 2022. From one-acre un-sprayed lemon orchards at district Naushahro Feroze, the population of the LBF natural enemies were observed and different infested stages of the pest population were collected on weekly basis. The pest-infested samples were frequently collected early in the morning hours of the day and the collection was kept in glass vials (1x5) inches in diameter; hence, brought to the Entomology Laboratory, Department of Zoology, Shah Abdul Latif University, Khairpur Mir's for identification and confirmation of natural enemies occurred under field conducted trials.

Laboratory examination

Until the presence of parasitoids, the infested and sluggish stages of the LBF population were kept separately for a series of treatments and new fresh *C. limon* leaves were given as the source of food as described methodology followed by (Rao *et al*., 2014). The diet of 1st, 2nd, and 3rd larvae was changed at an interval of 24 hours but the 4th and 5th larvae food was provided after 12 and 8 hours, respectively. When larvae converted into pupae they were transferred into new Petri dishes and maintained at 30.16°C (±0.30) at mean temperature with 65.5% (±4.50) relative humidity. On daily basis, the observation was taken on parasitoids that emerged from different developmental stages of the LBF. For the increasing life span of adult parasitoids, the cotton swab containing honey as nutritional supplement was kept inside the glass vials which were newly emerged from the *P. demoleus* eggs, larvae, and pupae as the naturally emerged parasitoids were recorded, respectively.

The hatching and confirmation of LBF egg parasitoids

The un-sprayed lemon orchards were found with a severe infestation at field conditions where the adult female LBF abundantly laid eggs on the tender twigs of plants. For the proper hatching and confirmation of parasitoid species, the branches containing up to ten infested leaves and twenty eggs were cut down up to 10cm and brought under laboratory examination. The branches were covered with cotton rolls and if supposed parasitoids hatched were observed the naked eyes were kept separated in vials and those vials were placed under 10kg plastic jars do not escape any parasitiod if hatched. If suppose to be, the parasitoids could not be hatched ultimately, the larvae emerged and started to overcome the leaves of the lemon twigs to prolong their life cycle smoothly. If suppose to be observed any egg parasitoids in minus or no appearance population in any week, it was ultimately increased in the second week, hence throughout the year data collection was made and formulated with Log^{10} formulation. The emerged population of parasitoids were converted into % population and the species were counted and kept separated after confirmation under laboratory examination and specimens were kept for future motivation.

The hatching and confirmation of LBF larvae parasitoids

From the un-sprayed lemon orchards, the collection of different larvae stages was made and brought to laboratory examination controlled at 25^{o}C and left for the emergence of the parasitoids. Intensive attention was given to natural enemies of each stage of larvae and if

wasp-like parasitoids were found kept separated for proper identification. For this twenty larvae were kept individually by giving fresh lemon leaves as a source of diet. Hence, the bulk culture was maintained and separated for proper identification, preservation, preparation of permanent slides, and pinning of the adult stages. The specimens were reserved for future perspectives.

The hatching and confirmation of LBF pupae parasitoids

The pupae population of the LBF was also collected from the un-sprayed lemon orchards and brought to the Entomology Laboratory for proper confirmation where the photographs were taken for future motivation. If the population of the parasitoids was found in excess again released under field conditions for the LBF population management as the trial basis and their re-emergence was kept under observation at selected lemon orchards. Hence; for the confirmation of parasitoid emergence, further treatments were replicated several times, as the photography was done, and if there was any critical appearance the help was taken through the help of stereoscopic microscope. For the proper identification of parasitoids, help was taken from Taxonomists keys, Entomological experts, and available literature, the parasitism percent population was calculated by the application under the given formula:

$$\text{Parasitism (\%)} = \frac{\text{No. of infected eggs, larvae, and pupae}}{\text{Total no emerged parasites from eggs, larvae, and pupae}} \times 100$$

Statistical analysis

The relationship among the population of the predators and parasitoids recovered from the eggs, larvae, and pupae of LBF were statistically analyzed. The least significant differences among the data of the parasite population was found at (P<0.05) through the application of the student package SXW software 8.1 USA, Origin 2017 64 Bit graphing & analysis software.

RESULTS

The population of ectoparasitoids and predators of LBF

The population of *P. demoleus*, predators, and parasitoids was observed and converted into the overall monthly mean population. Throughout the research period, the overall mean with standard deviation of lemon butterfly % population was counted (26.23±4.35) on un-sprayed lemon orchards and different species of predators including; *Aculepeira ceropegia* (Walckenaer, 1802), the maximum population of Oak spider recovered in April, September, October (0.25±0.05) and in the remaining months of the year at (0.05±0.22) with overall (0.10±0.31) mean population. During field examination ectoparasitiods namely; *Tamarixia radiata*, (Waterston, 1922) was found maximum in April, August, and October (0.50±0.70) followed by February, March, May, June, September, November, December (0.25±0.05), and remaining months of the year at (0.05±0.22) with overall (0.28±0.52) mean population. The *Tetrastichus* sp., (Haliday, 1844), belongs to the order; Hymenoptera and family; Eulophidae found maximum in May, September, and October (0.50±0.70) followed by January, February, March, April, June, July, August, November, December (0.25±0.05) individually and June (0.05±0.22) with overall (0.30±0.54) mean population. The maximum population of *Menochilus sexmaculatus* (Fabricus, 1781) was recorded in April, June, and August (0.25±0.05), and other remaining months of the year found up to (0.05±0.22) with overall (0.12±0.34) mean population. The maximum population of *Coccinella septempunctata,* (Linnaeus, 1758) was recorded in January, February, March, April, May (0.25±0.05), and in the remaining months of the year at (0.05±0.22) with overall (0.17±0.40) mean population. The maximum population of *Brumus* sp., was recorded in February, April, August, September (0.25±0.05) and in the remaining months of the year at (0.05±0.22) with an overall (0.12±0.33) mean on *Citrus limon* leaves.

The ANOVA between the *A. ceropegia* and pest population found (DF=1; F=1.02; P=0.48) with non-significant difference but found (DF=11; F=36.40; P=0.01) significant difference in the month-wise population of *A. ceropegia*. The *T. radiata* found (DF=1; F= 1.03; P=0.48) non-significant difference with pest population and (DF=11; F= 36.12; P= 0.02) significant difference in months throughout the year. The population of *Tetrastichus* sp., (DF= 1; F= 1.02; P= 0.48) within the pest and significant difference (DF= 11; F= 114.88; P= 048) in month-wise population. The *M. sexmaculatus* found non-significant difference (DF=1; F= 1.01; P= 0.49) in the pest population and significant difference (DF= 11; F= 36.25; P= 0.49) in different months of the year. The population of *C. septempunctata* found non-significant difference (DF=1; F= 1.01; P= 0.49) in pest insects and significant difference (DF= 11; F=

36.02; P= 0.03) in the months of the year. The *Brumus* sp., (DF= 1; F= 1.00; P= 0.49) non-significant difference with pest population and (DF= 11; F=36.17; P=0.05) significant difference between the *Brumus* sp., and different months of the year, respectively. The LSD shows the non-significant and significant difference (*P*<0.05) among the treatments, further justifications are shown in (Table 1).

The LBF larvae predators' population under field conditions

The overall maximum to the minimum monthly mean population of predators of different species including; *Peucetia viridans* (Hentz, 1832), recorded (0.25±0.05) in the March, May, September, and November followed by (0.05±0.22) in remaining months of the year with overall (0.012±0.34) mean. The *Cheiracanthium punctorium* (Villers, 1789), spider population was found individually in March, June, September, and October (0.25±0.05) and the remaining months (0.05±0.22) with overall (0.012±0.34) mean. The maximum mean population of *Halyomorpha halys* (Stal, 1855), bug species recorded at (0.25±0.05), in July, August, September, and November followed by (0.05±0.22) in the remaining months of the year individually. *Agelenopsis* sp., (Giebel, 1869) spider species was observed (0.25±0.05) in March, May, August, September, and in the remaining months, (0.05±0.22) with overall (0.012±0.34) mean. The maximum mean population of the *Delta pyriforme* (Fabricius, 1775), was recorded at (0.25±0.05) in April, July, August, September, and October and (0.05±0.22) in other months of the year with overall (0.13±0.36) mean. The maximum mean population of the *Geocoris punctipes* (Say, 1832), bugs counted in March, May, August (0.25±0.05), and the remaining months of the year at (0.05±0.22) with the overall (0.14±0.37) mean population recorded from the un-sprayed one acre *C. limon* orchards.

The analysis of the variance of *P. viridians* found the non-significant difference (DF= 1; F= 1.01; P= 0.49) in pest population and (DF= 11; F= 36.13; P= 0.05) significant difference in month-wise population.

Table 1. Monthly mean population of predators and parasitoids on *C. limon* leaves against *Papilio demoleus* under field conditions

Months	LBF	*Aculepeira ceropegia*	*Tamarixia radiata*	*Tetrasticus* sp.,	*Menochilus sexmaculatus*	*Coccinella septempunctata*	*Brumus* sp.,
Jan.	9.75±3.12	0.05±0.22^{b}	0.05±0.22^{b}	0.25±0.05^{b}	0.05±0.22^{b}	0.25±0.05^{b}	0.05±0.22^{b}
Feb.	19.50±4.42	0.05±0.22^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}
Mar.	31.00±5.57	0.05±0.22^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}
Apr.	34.00±5.83	0.25±0.05^{ab}	0.50±0.70^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0. 25±0.05^{ab}	0.25±0.05^{ab}
May	27.00±5.20	0.05±0.22^{ab}	0.25±0.05^{ab}	0.50±0.70^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}
Jun.	11.50±3.39	0.05±0.22^{b}	0.25±0.05^{b}	0.05±0.22^{b}	0.25±0.05^{b}	0.05±0.22^{b}	0.05±0.22^{b}
Jul.	15.50±3.94	0.05±0.21^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.05±0.22^{ab}	0.05±0.22^{ab}
Aug.	36.00±6.00	0.05±0.22^{ab}	0.50±0.70^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}
Sep.	39.00±6.24	0.25±0.05^{ab}	0.25±0.05^{ab}	0.50±0.70^{ab}	0.05±0.22^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}
Oct.	60.25±7.76	0.25±0.05^{a}	0.50±0.70^{a}	0.50±0.70^{a}	0.05±0.22^{a}	0.25±0.05^{a}	0.05±0.22^{a}
Nov.	23.50±4.85	0.05±0.22^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}
Dec.	7.75±2.78	0.05±0.22^{b}	0.25±0.05^{b}	0.25±0.05^{b}	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}
Mean±SD	26.23±5.12^{a}	0.10±0.31^{b}	0.28±0.52^{b}	0.30±0.54^{b}	0.12±0.34^{b}	0.17±0.40^{b}	0.12±0.34^{b}

Each value is the mean±SD of predators and parasitoids population of LBF

Table. 2. Monthly mean population of predators on *C. limon* leaves against *Papilio demoleus* at field conditions

Months	LBF	*Peucetia viridans*	*Cheiracanthium punctorium*	*Halyomorpha halys*	*Agelenopsis sp.,*	*Delta pyriforme*	*Geocoris punctipes*
Jan.	9.75±3.12	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}
Feb.	19.50±4.42	0.05±0.22^{ab}	0.05±0.22^{ab}	0.05±.022^{ab}	0.05±0.22^{ab}	0.05±0.22^{ab}	0.05±0.22^{ab}
Mar.	31.00±5.57	0.25±0.05^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}
Apr.	34.00±5.83	0.05±0.22^{ab}	0.05±0.22^{ab}	0.05±0.22^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}
May	27.00±5.20	0.25±0.05^{ab}	0.05±0.222^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.05±0.05^{ab}
Jun.	11.50±3.39	0.05±0.22^{b}	0.25±0.05^{b}	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}
Jul.	15.50±3.94	0.05±0.22^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}
Aug.	36.00±6.00	0.05±0.22^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}
Sep.	39.00±6.24	0.25±0.05^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}
Oct	60.25±7.76	0.05±0.22^{a}	0.25±0.05^{a}	0.05±0.22^{a}	0.05±0.22^{a}	0.25±0.05^{a}	0.50±0.70^{a}
Nov.	23.50±4.85	0.25±0.05^{ab}	0.05±0.22^{ab}	0.25±0.05^{ab}	0.05±0.22^{ab}	0.05±0.22^{ab}	0.05±0.22^{ab}
Dec.	7.75±2.78	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}	0.05±00.22^{b}
Mean±SD	26.23±5.12^{a}	0.12±0.34^{b}	0.12±0.34^{b}	0.12±0.34^{b}	0.12±0.34^{b}	0.13±0.36^{b}	0.14±0.37^{b}

Each value is the mean±SD of the predator's population on LBF

Table 3. Parasitoids monthly mean population % recovered from eggs of LBF under laboratory conditions

Months	Egg parasitoids			
	Eggs	***Ooencyrtus papilionis* %**	***Telonamus* sp.%**	***Trichogramma chilonis* %**
Jan.	4.50±2.12	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}
Feb.	8.00±2.38	0.05±0.22^{ab}	0.05±0.22^{ab}	0.14±0.37^{b}
Mar.	13.25±3.64	0.10±0.31^{ab}	0.05±0.22^{ab}	0.30±0.55^{ab}
Apr.	14.25±3.77	0.05±0.22^{ab}	0.21±0.46^{ab}	0.29±0.53^{ab}
May	11.75±3.43	0.05±0.22^{ab}	0.09±0.30^{ab}	0.21±0.45^{ab}
Jun.	5.25±2.29	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}
Jul.	6.75±2.60	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}
Aug	15.25±3.91	0.15±0.39^{ab}	0.19±0.44^{ab}	0.23±0.48^{ab}
Sep.	15.75±3.97	0.16±0.39^{ab}	0.20±0.44^{ab}	0.32±0.56^{ab}
Oct.	28.75±5.36	0.43±0.65^{a}	0.29±0.54^{a}	0.86±0.93^{a}
Nov.	10.25±3.20	0.05±0.22^{ab}	0.05±0.22^{ab}	0.18±0.42^{ab}
Dec.	3.50±1.87	0.05±0.22^{b}	0.05±0.22^{b}	0.05±0.22^{b}
Mean±SD	11.44±3.38^{b}	0.10±0.32^{a}	0.11±0.33^{b}	0.23±0.48^{a}

Each value is the parasitoids % population from the infested eggs of LBF

Table 4. Parasitoids monthly mean population % recovered from the larvae and pupae of the LBF under laboratory conditions

Months	Larvae parasitoids			Pupae parasitoids	
	Larvae	***Apanteles papilions* %**	***Distatrix papilionis* %**	**Pupae**	***Pteromalus puparum* %**
Jan.	2.50±1.58	0.05±0.22^{b}	0.05±0.22^{b}	1.25±1.12	0.05±0.22^{ab}
Feb.	5.25±2.29	0.05±0.22^{ab}	0.05±0.22^{ab}	2.75±1.66	0.01±0.12^{ab}
Mar.	8.25±2.87	0.04±0.20^{ab}	0.14±0.38^{ab}	5.50±2.35	0.08±0.29^{ab}
Apr.	9.00±3.00	0.25±0.50^{ab}	0.05±0.22^{ab}	6.25±2.50	0.14±0.38^{ab}
May	7.00±2.65	0.04±0.19^{ab}	0.09±0.30^{ab}	4.25±2.06	0.04±0.21^{ab}
Jun.	2.75±1.66	0.05±0.22^{b}	0.05±0.22^{b}	1.50±1.22	0.05±0.22^{ab}
Jul.	3.75±1.94	0.05±0.22^{b}	0.01±0.10^{b}	2.00±1.41	0.05±0.22^{ab}
Aug	9.75±3.12	0.07±0.27^{ab}	0.22±0.47^{ab}	6.50±2.55	0.16±0.40^{ab}
Sep.	10.50±3.24	0.16±0.40^{ab}	0.24±0.49^{ab}	7.75±2.78	0.23±0.48^{ab}
Oct.	16.75±4.09	0.21±0.46^{a}	0.59±0.77^{a}	9.25±3.04	0.35±0.59^{a}
Nov.	6.25±2.50	0.09±0.31^{ab}	0.05±0.22^{ab}	3.50±1.87	0.03±0.16^{ab}
Dec.	2.00±1.41	0.05±0.22^{b}	0.05±0.22^{b}	0.75±0.87	0.05±0.22^{b}
Mean±SD	6.98±2.64^{b}	0.09±0.30^{a}	0.13±0.36^{b}	4.27±2.07^{b}	0.10±0.32^{a}

Each value is the parasitoids % population from the infested larvae and pupae of LBF

The *C. punctorium,* (DF=1; F=1.01; P= 0.49) was found non-significant with pest and significant difference (DF=11; F=36.25; P= 0.03) in the months of the year. *H. halys* found (DF=1; F=1.00; P=0.49) non-significant with insect pest and (DF=11; F=36.09; P=0.04) significant difference in months. *Agelenopsis* sp., (DF=1; F=1.01; P=0.49) non-significant with pest population and (DF=11; F=36.20; P=0.02) significant difference with month-wise population. *D. pyriforme*, (DF=1; F=1.02; P= 0.48) non-significant difference with LBF population and (DF=11; F=36.00; P=0.01) significant difference in months. *G. punctipes* was found with the non-significant difference (DF=1; F=1.03; P=0.48) in the pest population and significant difference (DF=11; F=36.51; P= 0.01) in month wise population of the ectoparasitiods on the *C. limon* leaves. The difference between the pest and predators' population was counted (P<0.05), and further justification of the species population was described in (Table 2).

Lemon butterfly eggs parasitoids population under laboratory conditions

Under laboratory conditions, the population of the parasitoids was recovered from the infested egg of the LBF. The maximum to the minimum mean population of the parasitoids was collected at (28.75±5.36) in October followed by (15.75± 3.97) September, (15.25±3.91) August, (14.25± 3.77) April, (13.25±3.64) March, (11.75±3.43) May, (10.25±3.20) November, (8.00±2.38) February, (6.75±2.60) July, (5.25±2.29) June, (4.50±2.12) January, (3.50±1.87) December, with overall (11.44±3.38) mean and SD. The maximum to minimum percent population of the egg parasitoids; *Ooencyrtus papilionis* counted in October (0.43±0.65) followed by September (0.16±0.39), August (0.15±0.39), March (0.10± 0.31), and (0.05±0.22) in other months of the year with (0.10±0.32) overall mean. The

population of *Telenomus* sp., recorded in October (0.29±0.54), April (0.21±0.46), September (0.20±0.44), August (0.19±0.44), May (0.09±0.30), and in rest months of the year (0.05±0.22) with (0.11±0.33) overall mean. The population of *Trichogramma chilonis* calculated in October (0.86±0.93) (0.86±0.93) followed by September (0.32±0.56), March (0.30±0.55), April (0.29±0.53), August (0.23±0.48), May (0.21± 0.45), November (0.18±0.42), February (0.14± 0.37), and (0.05±0.22) in January, June, July, and December with (0.23±0.48), respectively.

The ANOVA of infested eggs and recovered parasitiod, *O. papilionis* was found non-significant difference (DF=1; F=1.06; P=0.46) with pest population and significant difference (DF=11; F=33.00; P=0.04) in month wise insect pest population. *Telenomus* sp., with the non-significant difference (DF=1; F=104; P= 0.47) in pest population and significant difference (DF= 11; F= 2.74; P=0.02) in the month wise of the year. The parasitiod, *T. chilonis* found to non-significant difference (DF=1; F=1.13; P=0.41) with the pest eggs population and (DF=11; F= 33.43; P=0.03) significant difference month-wise population of the insect pest, validation shown in (Table. 3).

LBF larvae and pupae parasitoids population under laboratory conditions

Under laboratory conditions, the population of the larvae and pupae was observed for the parasitoids prevalence. The maximum to minimum percent infestation with SD in larvae of the insect pest was recorded (16.75±4.09) in October followed by (10.50±3.24) September, (9.75±3.12) August, (9.00±3.00) April, (8.25± 2.87) March, (7.00±2.65) May, (6.25±2.50) November, (5.25±2.29) February, (3.75±1.94) July, (2.75±1.66) June, (2.50±1.58) January, (2.00±1.41) December, with (6.98±2.64) overall mean and SD. The maximum to minimum parasitiod population of larvae such as; *Apanteles papilions* was found in April (0.25±0.50) followed by October (0.21±0.46), September (0.16±0.40), November (0.09±0.31), August (0.07±0.27), January, February, June, July, December (0.05±0.22), March (0.04±0.20) and May (0.04±0.19) with (0.09±0.30) overall mean. The *Distatrix papilionis* in October (0.59±0.77), followed by September (0.24±0.49), August (0.22±0.47), March (0.14±0.38), May (0.09±0.30), January, February, April, June, November, December (0.05±0.22), and (0.01± 0.10) in July, with (0.13±0.36) overall mean, respectively. Simultaneously, the maximum to minimum percent and SD infestation in pupae of the LBF was counted (9.25±3.04) in October, followed by (7.75±2.78) September, (6.50±2.55) August, (6.25±2.50) April, (5.50±2.35) March, (4.25±2.06) May, (3.50±1.87) November, (2.75± 1.66) February, (2.00±1.41) July, (1.50±1.22) June, (1.25±1.12) January, (0.75±0.87) December, with (4.27±2.07) overall mean and SD. The maximum parasitiod population i-e., *Pteromalus puparum* recorded in October (0.35±0.59) followed by September (0.23±0.48), August (0.16±0.40), April (0.14±0.38), March (0.08±0.29), January, June, July, December (0.05±0.22), May (0.04±0.21), November (0.03± 0.16), and February (0.01±0.12), with (0.10± 0.32), respectively.

The ANOVA and LSD test of the larvae parasitiod, *A. papilions* statistically analyzed found non-significant difference (DF= 1; F=1.05; P=0.46) in the pest population and significant difference (DF=11; F=32.39; P=0.03) in monthly parasitoids mean population. The larvae parasitiod, *D. papilionis* found non-significant difference (DF=1; F=1.14; P=0.41) with insect pest population and significant difference (DF= 11; F=33.47; P=0.03) in months of the year. The *P. puparum* population found non-significant difference (DF=1; F=1.13; P=0.41) with pest population and significant difference (DF=11; F=28.89; P=0.04) in month-wise population as validation given in (Table. 4).

DISCUSSION

During the research study, intensive attention was given to the population of the lemon butterfly and their general predators from one acre of un-sprayed lemon orchards. The population of Oak spider, *Delta pyriforme*, *Geocoris punctipes,* and *Halyomorpha halys* were recovered throughout the year on *C. limon* as the work shows the similarity with (Suwarno, 2012) who reported the Stink bugs, Chalcid wasp, and spiders caused mortality of eggs and larvae of LBF. The population of *Tamarixia radiata* was reported on the leaves of lemon plants, same findings were also reported by Benson *et al*. (2003). The population of parasitiod; *Tetrastichus* sp., was observed with as the already reported work of Debbarma and Hath (2021) who found that the coccinellid predators, hymenopteran parasitoids, and spiders from the lemon cultivated orchards. The population of *Peucetia viridans, Cheiracanthium punctorium, Agelenopsis* sp., and *Menochilus sexmaculatus* were found with the work agreement of (Santhosh and Basavarajappa,

2017) who documented the *Argiope*, *Hippasa*, *Zygeila*, *Rhene*, *Selenops* spiders species of predators and parasites of the eggs, larvae, pupae, and adults of LBF. The population of *Coccinella septempunctata* and *Brumus* sp., observed with the agreement of (Rothe *et al.,* 2019) who reported ladybird beetles from citrus plants.

Similarly, the population of endoparasitiods namely; *Ooencyrtus papilionis* recovered from the infested eggs of LBF as the same species of parasitiod; *Trichogramma* sp., *Ooencyrtus papilionis,* and *Telenomus* sp., of parasitoids reported from the egg of LBF (Jalali and Singh, 1990). The *A*. *angaleti*, *T*. *chilonis*, *Telenomus* sp., and *O*. *papilionis* parasitoids were documented from the host *C*. *reticulata*, *C*. *sinensis,* and *C*. *aurantiifolia* (Bhapkar *et al*., 2015). The population of *Telenomus* sp., reported from the eggs of LBF is more or less comparable reported work of (Thakare and Borle, 1974) who documented the *T. chilonis* and *Telenomus* sp., from the eggs of LBF. *Trichogramma*, *Apanteles*, *Habrobracon hebetor* and *Telenomus* caused 73% parasitism on *P. demoleus* and *P. polytes* species (Sarada *et al*., 2014) among them *A. paplionis* parasites with maximum mortality % (Dadmal, 2004). The population of egg endoparasite *Trichogramma chilonis* observed with the work similarity of (Delano, 2018) reported egg parasitoids, *Ooencyrtus malayensis*, *Tetrastichus* sp., and *A*. *paplionis*, *Distatrix papilionis*, *P*. *puparum*, *Apanteles*, *B*. *hebetor* the larvae and pupae parasitoids were recorded from the lemon butterfly. *Apanteles papilions* endoparasite recovered from the larvae of the LBF same findings of (Resham *et al*., 1986) reported *Apanteles lunatus* and *Pteromalus puparum* parasites reported from the larvae of LBF. (Gupta and Pereira, 2012) documented Glyptapanteles *hypermnestrae* parasitiod wasps found the larvae of *Elymnias hypermenestra,* also *A. folia* and *B. indica* first time from the pupae and larvae of the *Arhopala amantes* butterfly. Another species of endoparasite LBF larvae *Distatrix papilionis* found with the comparable work of Bhoje and Charaple, (2020) reported the first time *A. papiliones* form the larvae of the lemon butterfly in Maharashtra, India.

The endoparasite *Pteromalus puparum* recovered from the pupae of LBF as documented by Ramzan and Darshan, (1979) pupae parasite, *Pteromalus* sp, praying mantids, spiders, yellow wasp, at larvae stage and hymenopterans parasite form the egg of LBF. (Bartel *et al*., 2011) recovered *Brachymeria* sp., and *Pteromalus puparium* from the pupae of *P. demoleus* in North America. Gupta and Haldhar, (2012) reported *Brachymeria indica* wasp and *Brachymeria jambolana* pupal parasitiod from the pupae of *Pareronia valeria* and *Delias eucharis* host species. Ahmed *et al*. (2014) reported *Sturmia convergens* from the pupae of *Danaus chrysippus* butterfly species Sarada *et al*. (2014) documented *Polistes hebraeus* and *Creobroter gemmatus* mantis predators, and *Apanteles* sp., *Holcojoppa coelopyga*, *Brachymeria*, *H*. *hebetor*, *P*. *puparium*, *O*. *paplionis*, *Erycia nymphalidophaga*, *A*. *flavipes*, *Tetrastichus sp*., and *Melalophacharops* sp., were recovered from the eggs, larvae, and pupae of the LBF.

There is a common nature in parasitoids to live in or on the larvae and finally kill their host by consuming the vital body organs it has been estimated that about more than 10% of species of insects are parasitoids and different other species such as; *Eocanthecona furcellata*, Pentatomid bugs, Reduviid bugs, Spiders, Birds, Chameleons, and Sphecids are the biological controlling agents of the butterflies (Eggleton and Balshaw, 1992). There are several control practices are available for growers to introduce and enhance biological control strategies because many species of natural enemies are easily available in commercial fields and gardens. As *P. demolues* there are several other pest insect species causing severe problems in lemon orchards and their fruits should be kept for proper management in future perspectives. From the given Sahati region of Sindh-Pakistan, no such type of research work has been conducted previously. Therefore, the application of different insecticides is the first attempt against the larvae of LBF. Hence, it is an immediate call and dire need for more studies should be carried out on the parasitoid species interaction with the population of LBF.

CONCLUSION

The application of pesticides was also prohibited under the lemon orchard and its vicinity for research purposes. It was concluded that the pest insect *P. demoleus* vigorously damaging to *C. limon* leaves but different species of predators and parasitoids served as biological controlling agents. On the basis of our findings, it is recommended that the knowledge of parasitoids associated with their host species, eco-friendly measures, and conservation of

natural enemies with holistic approach is immediately needed. It is further recommended to culture the population of parasitoids conserve and release the biological controlling agents to citrus orchards and minimize the application of toxic insecticides.

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AUTHOR'S CONTRIBUTION

W. M. Mangrio: Designed and conducted the research and wrote the manuscript.
H. A. Sahito: Designed and supervised the research work.
F. I. Sahito: Statistical analysis and data compilation.
F. A. Jatoi: Manuscript revision and proof reading.
N. H. Jakhrani: Helped in species identification.

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