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Effect of Various Hosts on The Biology of Trichogramma Chilonis (Hymenoptera: Trichogamatidae) Under Lab Conditions.

[1]Farman Ali, [2]Habib Ahmed Khosa, [3]Muhammad Sharif, [4]Abdul Jabbar,[5]Shah Muhammad Khosa, [6]Sadam Hussain.

1.Agriculture officer (usta Muhammad)Agriculture extension Balochistan 87300, Pakistan
2.Agronomist DAR Sibi Agriculture Research Balochistan, Pakistan.
3.Agriculture officer Agriculture extension Balochistan, Pakistan
4.Agriculture officer Agriculture extension Balochistan, Pakistan[2]
5.Entomologist DAR Plant Protection ARI , Quetta, 87300, Pakistan.
6.Marine Specialist Balochistan, Pakistan

Abstract : The present studies on the effect of various hosts on the biology of *Aganaspis daci* previously known as *Trichogramma chilonis (Hyenoptera:trichogrammatidae)*now known as Eucoilidae) were carried out in the fruitfly and their parasitoids lab, Plant Protection Division, Nuclear Institute of Agriculture (NIA), Tandojam. Four various kinds of hosts namely, *Bactrocera zonata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae* and *Carpomya vesuviana* were used in the experiment. Data on number of un-emerged, parasitized pupae, sex ratio and longevity of the parasitoid were recorded.

The various parameters of *T.chilonis*were significant (P<0.05) affected by the provision of various hosts. However, maximum mean parasitization was recorded when *T.chilonis*were offered with the larvae of *B. zonata* (11.40±0.96) and minimum in *C. vesuviana* (4.60±0.96). Moreover, maximum mean emergence was observed when *T.chilonis*were offered with the larvae of *B. zonata* (8.90±0.87) and minimum in *C.vesuviana* (3.30±0.94). Moreover, maximum male emergence were recorded when *T.chilonis*were offered with the larvae of *B. zonata* (3.70±0.50) and lowest in *C. vesuviana* (1.00±0.21). Similarly maximum mean female emergence was recorded, when adults *T.chilonis*were offered with larvae of *B. zonata* (5.20±1.45) and maximum in larvae of *C. vesuviana* (2.00±0.87). Our results established that *B. zonata* is preferred host of *T.chilonis*in term of preference, parasitization, emergence and longevity. These results could be exploited for the mass rearing and management of *B. zonata* in variety of orchard agro-ecosystem.

Keywords: Effect of various hosts on the biology of *Trichogramma chilonis* under lab conditions

INTRODUCTION:

Fruitflies in the family Tephritidae are high profile insects among commercial fruit and vegetable growers, marketing exporters, government regulatory agencies, and the scientific community. Locally, producers face huge losses without some management scheme to control fruit fly populations. (McPheron and Steck 1996) Fruit flies attack fruits and vegetables and not only reduce their yield but also affect the quality. Damage to fruits cause loss of about 7 billion rupees to farmers annually in Pakistan besides the losses to traders, retailers and exporters. The host fruits and vegetables attacked by fruitflies like *Bactrocera zonata* and *Bactrocera dorsalis* include guava, plum, peach, apricot, loquat, bitter gourd, citrus, mango, sponge gourd and pear. Some fruits such as guava were severely damaged by fruit flies causing up to 100% loss of harvested fruits at Huripur, Kohat. In Pakistan, sole reliance has been made on pesticides for the control of fruit flies which has created environmental contamination, residues problem, killing of non-target organisms, development of resistance against pesticides in insects etc, however, in some instances, the use of

male lures and protein baits are being fostered for the control of fruitfly. These control measures are not practiced in integrated manner (Khan, 1997). Among various species of pest insects attacking the fruits, fruitflies (Tephritidae: Diptera) have great economic importance due to their heavy losses to fruits in Pakistan. The incidence of fruitflies reduces both yield and quality of fruits when females puncture and lay eggs. The larvae or maggots after hatching tunnel into the fruits for feeding on the pulp and render them unfit for human consumption. In certain fruits, rotting starts at the puncture points. The losses caused to fruits by fruit flies varied according to species and the host fruit plant species. In the scientific literature the most serious pest species reported is the oriental fruit fly (*B.dorsalis*). Highest loss of 80 percent in guava fruit was reported by Jalaludin *et al.* (1999) reported 60-80 percent loss in guava fruit by *Bactrocera correcta* (Bezzi). The peach fruitfly (*B.zonata*) is another insects pest species found most abundantly in all climatic regions of Pakistan and cause 3-100 percent loss in defferent fruits.The ber fruitfly (*Carpomya vesuviana)* can cause 90-100 percent damage to ber fruit (Kapoor, 1993). Fruitflies are controlled by various techniques such as male annihilation technique (MAT) with methyl eugenol baited traps. It has been very successful in eradication of oriental fruitfly from Rota Island, Amami Island and Okinawa Island. Sterile insect technique (SIT) was also used to eradicate *B.dorsalis* from the Ogasawara Islands and *B.cucurbitae* from Kume Island, Japan (Shiga, 1989).

The farmers spray toxic chemicals on vegetables, oilseeds and fruit crops in order to avoid the pest infestation. Due to the intensive and indiscriminate use of many pesticides' poison, people suffer from many diseases, and some of these are chronic for human beings. Use of pesticides has resulted in the environmental pollution on large scale. Besides contaminating food and food products, pesticides have been accumulating in the soil, air and water to a critical stage. This calls for a safe and cheap control method of these insect pests; and which can only be achieved by the practice of Integrated Pest Management (IPM); a pest control management which ensures environmental safety (Solangi, 2004).

Classical biological control of tephritid fruitfly by pests using parasitoids has been successful in a few subtropical and tropical regions (Wharton 1997, Purcell 1998, Ovruski 2000). Biological control in this regard occupies a central position in Integrated Pest Management (IPM) Programs. Because biological control of invertebrate pest and weeds has enormous and unique advantages, it is safe, permanent and economical. *Trybliographa daci (*Weld) was first collected in Malaysia and Borneo, and introduced into Hawaii as a potential biocontrol agent for *B. dorsalis* (Hendel). Biological control of fruit flies has been attempted mainly with braconid parasitoids (Hymenoptera). In several parts of the world, species of the genus *Diachasmimorpha* (5 *Biosteres* 5 *Opius*) have been introduced for the classical biological control of these pests (Wharton, 1997). Augmentative biological control, the mass release of parasitoids at appropriate times and places, has been proposed as a new approach for fruitfly suppression (Knipling, 1992)

REVIEW OF LITERATURE

Glenn *et al*. (1987) determined that the approximately 4,000 *Trybliographa daci* (Weld) were received in 1977 from Dr. R. Pralavorio, This species is indo-Australasian in origin, most commonly using *Dacus sp.* As hosts. It was reared in the INRA laboratory on *Ceratitis captata* (Weld.). During the period 1979-1981, 43,310 individuals were released in Dade County, FL. Although a considerable number of fruit samples have been taken from the release sites no recoveries were made until the summer of 1984. A sample of 500 Surinam cherries (*Eugenia uniflora* L.)

collected on 18-V-84 at the Tropical Research and Education Center yielded 488 *A. suspense* pupae from which 7 *T.chiloni*semerged. A sample of 55 guavas (*Psidium littorale var longipes* (O. Berg) Fosb. Collected at the same location on 17-VII-84 yielded 163 A *suspense* pupae from which 2 *T.chiloni*semerged. In addition, 4 males and 17 females were recovered from *A.suspensa* larvae that emerged from 10 *Psidium guanava* L. collected on 25-VII-85; 12 males, 2 females from the same host collected on 28-VIII-85; 1 female from the same host out of 4 *P. guajava* collected on 13-IX-85; and 10 males and 45 females were recovered from 479 *A. suspense* larvae that had emerged from 10 *P. guajava* collected on 16-X-85, all from the same locality.

Gupta *et al.* (1990) studied seasonal fluctuation of *Dacus zonatus* (*Bactrocera zonatus*) and *D. dorsalis* (*B. dorsalis*) in semi-isolated peach, plum and apricot orchards located in the mid-hill region of Himachal Pradesh, India during 1986 and 1987, traps baited with methyl eugenol and malathion captured males from both species from the 2nd week of April until the 2nd week of November during both years, although more *B. zonatus* adults were caught. In 1986, peak adult activity occurred during the 3rd week of June on apricot, the 4th week of June on plum and the 2nd week of July on peach. A similar trend occurred in 1987; however the maximum catch on peach occurred during the 3rd week of June.

Aluja *et al.* (1990) represented the total no of 1,302 parasitoids from 8 species and 4 families were recovered from 9,818 fruit fly host fruits sampled. The most common parasitoid species was *Diachasmimorpha longicaudata* (Ashmead). Average percent parasitism ranged between 0.44 and 29.23 %. Parasitoid emergence data indicate that *Anastrepha ludens* (Loew), *A. obliqua* (Sein), *A. serpentina* (Wiedeman), *A. striata* (Schiner) and *Toxotrypana curvicauda* (Gerstaecker) were subject to parasitism. We provide information on the population fluctuation of *Anastrepha ludens, A. obliqua, A. serpentina, A. distincta* (Greene), *A. striata, A. fraterculus* (Wiedeman), *A. chiclayae* (Greene), *A. montei* (Costa Lima), *A. leptozona* (Hendel) and *A. tripunctata* (Wulp). *Anastrepha ludens* and *A. obliqua* were the most common species, representing 95.3 % of all fruit fly species caught in McPhail traps.

Liu and Chen (1992) studied attractiveness of three proteinaceous substances (protein hydrolysate, Nu-lure and PIB-7 to *B. dorsalis* and reported that protein hydrolysate was the most effective bait attracting 45.4% of adult females and 35.6% of males.

Baranowski *et al.* (1993) introduced parasitic hymenoptera into Florida in an attempt to bring the Caribbean fruitfly (caribfly) under biological control. A total of 15 species of parasites from four families were imported twelve species were released, nine have been recovered in the field, and five are considered established. These coexist with both endermic fruitfly parasites and generalistspecies, which serendipitously attack caribfly. Inundative releases of the brconid *Diachasmimorpha longicaudata* to control caribfly are presently being tested. It is hypothesized that releases of parasites will augment numbers of natural enemies during periods when wasps are relatively uncommon due to difficulties in host finding. The lower numbers of flies that may result could be important in creating and maintaining fly-free zones. A renewed interest in the biological control of fruitflies promises future explorations for new natural enemies and novel means of employing them.

Cohen *et al*. (1995) reported that the fruit flies have been the subject of experimentation for many years. High fruit fly populations and the abundance of fruits throughout the year contribute to the status of fruit flies as the major pest of cultivated fruits. Fruit flies can be controlled effectively by regular applications of cover sprays using insecticides; however, this method of control may be detrimental to beneficial insect fauna such as predators, parasitoids, bees and other pollinators, and could also cause environmental pollution.

Allwood and Drew (1996) reported that the seasonal activity of *Dipterophagus daci* was dependent on the availability of its host and rainfall. They further reported that the population increased with the onset of higher temperatures and moisture level.

Mahmood *et al.* (1996) determined in Pakistan the fruit flies of economic importance are *Bactrocera dorsalis, B. zonata*, *B. cucurbitae*, *Dacus ciliatus* and *Myiopardalis pardalina*. The information on distribution, biology, population build-up, host and host fruit relations of parasitoids of fruit flies was collected. *Biosteres longicaudatus* a common parasitoid of *Bactrocera zonata* and B. dorsalis is widely distributed in Pakistan. Various races of *Biosteres longicaudatus* from Karachi (coastal areas) completed development in *Bactrocera zonata* only whereas the population from Haripur and Lahore completed development both in *B. zonata* and B. dorsalis. *Trybliographa daci* is a dominant parasitoid of *B. zonata* in the plains, the coastal and sub-coastal areas. *Dirhinus giffardii* is a common parasitoid of *Dacus ciliatus* in sub-coastal areas and *Spalangia sp*. of *B. cucurbitae* in the plains of Pakistan. No parasitoid was recorded from *Myiopardalis pardalina*.

MATERIALS AND METHODS:

Insect Colony and hosts

Studies were carried out on effect of various hosts on the biology of *Trybliographa daci* (Hymenoptera: Brachonidae) in the fruit fly and their parasitoids laboratory, Plant Protection Division, Nuclear Institute of Agriculture (NIA) Tandojam. For the experiment larval parasitoid *Trybliographa daci* was obtained from the parasitoid rearing laboratory. Moreover, the hosts *Bactrocera zonata*, *Bactrocera dorsalis,* and *Bactrocera cucurbitae* were obtained from the fruit fly laboratory. However, Ber fruit fly *Carpomya vesuviana* was collected and reared from the collection of infested fruit. All the hosts were reared in the laboratory and then offered to *T. daci,* and were maintained in cages (3 x 1.5 x 3 ft). Adults of the fruit flies had access to sugar, protein hydrolysate, casein and water soaked. Maggots of fruit flies were reared in enamel trays and were provided with mixed diet of wheat shorts, sugar, yeast, methy para hydro-oxy benzoate, sodium benzoate and Hcl. Pupae were collected and sieved from pupal substrate (2.5 x 3 x 2.5 ft). Rearing conditions for stock cultures of fruit flies were 25 ± 2°C and a photoperiod of 16L: 8D, 65±5% RH.

Four pairs of the *T.daci* were released in the cage and afterwards 2nd instars of the each host larvae were placed in Petri dishes containing artificial diets for 24 hours in each of jar. After 24 hours larvae were reared as mentioned for colony maintenance Pupae were collected and sieved from pupal substrate the pupae were kept for recording the counting adult emergence. Emerged an adults were kept in jars to record their longevity. Adult parasitoids were provided with honey as described above for colony.

To study the host suitability

Four kinds of hosts namely *B.zonata, B.dorsalis, B. cucurbitae* and *C.vesuviana* were offered to *T.chilonis*for 24 hour. Each pair of *T.chilonis*was kept in separate jar. Forty larvae of the each host were offered to the parasitoid upto the female natural death. Observation on parasitization, weight, width and length of parasitized pupae, number of emerged adults and sex ratio was recorded.

Effect of various hosts on the longevity and fecundity of *T.daci*

Freshly emerged adults of *T.chilonis*were paired and kept in cages (26 x 20 x 23 cm) and were provided with adult diet as described above for colony maintenance. To examine fecundity and fertility of *T.chilonis*fed with various host larvae were provided upto mortality of the female. Males were replaced with new males. Observation on adult longevity, fecundity and fertility were recorded. In each treatment 15 pairs of the *T.chilonis*were tested. The temperature and humidity were maintained as described above for colony.

RESULTS:

The present study were carried out to evaluate the effect of various hosts on the biology of *Trybliographa daci* (Hymenoptera: Eucoilidae) in the fruitfly and their parasitoids laboratory, Plant Protection Division, Nuclear Institute of Agriculture (NIA) Tandojam. For the experiment larval parasitoid *Trybliographa daci* was obtained from the parasitoid rearing laboratory. Moreover, the hosts *Bactrocera zonata, Bactrocera dorsalis,* and *Bactrocera cucurbitae* were obtained from the fruitfly laboratory. However, Ber fruitfly *Carpomya vesuviana* was collected and reared from the collection of infested fruit. The data were recorded on number of unemerged and parasitized pupae, total number of emerged parasitoid, total number of male parasitoid and total number of female parasitoid. The results on these parameters were presented in figure-1 to 7 and appendices.

Effect of various hosts on parasitization of *T.chilonis*

The parasitization by the *T.chilonis*on various hosts was significantly differed ($P < 0.05$) as represented in Fig 1. Results revealed that maximum parasitization (11.40±0.96) was documented in *B. zonata* followed by *B. dorsalis,* and *B. cucurbitae* (8.90±1.19 and 6.70±0.94), respectively. However, minimum parasitizations were recorded in *C. vesuviana* (4.60±0.96).

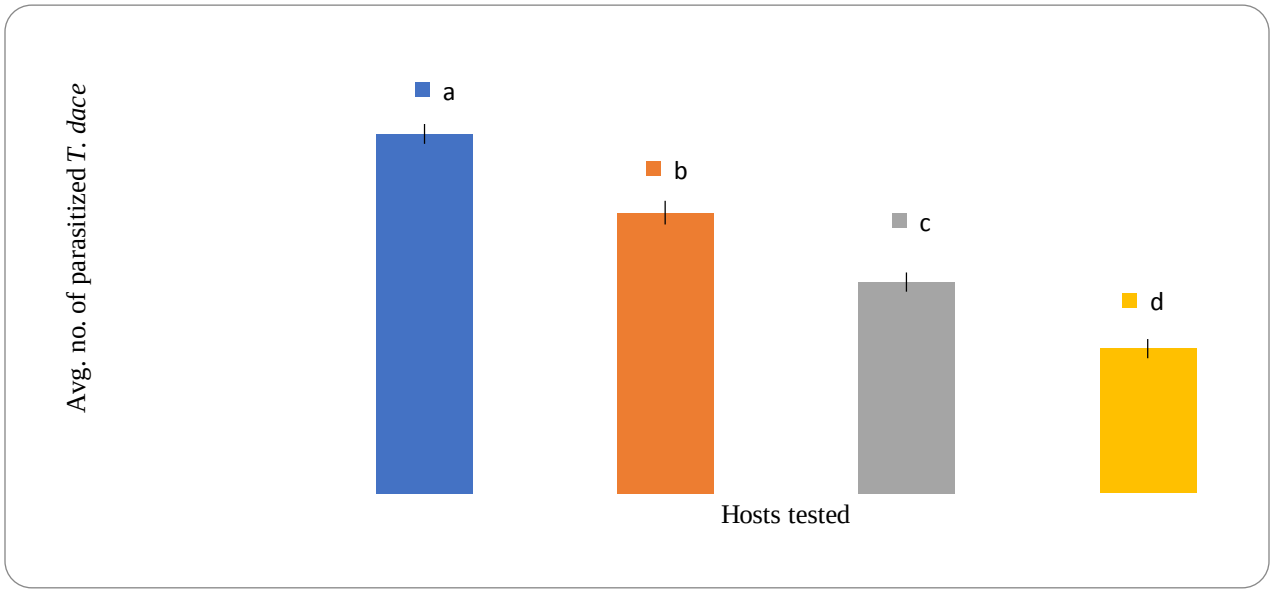

Fig. 1. Mean number of parasitized pupae of various hosts under laboratory conditions.

Effect of various hosts on emergence of *T.chilonis*

The emergence of the parasitoids from various hosts were significantly ($P < 0.05$) affected as shown in Fig. 2. Results revealed that maximum number of emerged adults (8.90±0.87) was recorded in *B. zonata* followed by *B. dorsalis*, and *B. cucurbitae* (6.60±1.17 and 4.80±1.30), respectively. However, minimum numbers of emerged adults were recorded in *C. vesuviana* (3.30±0.94).

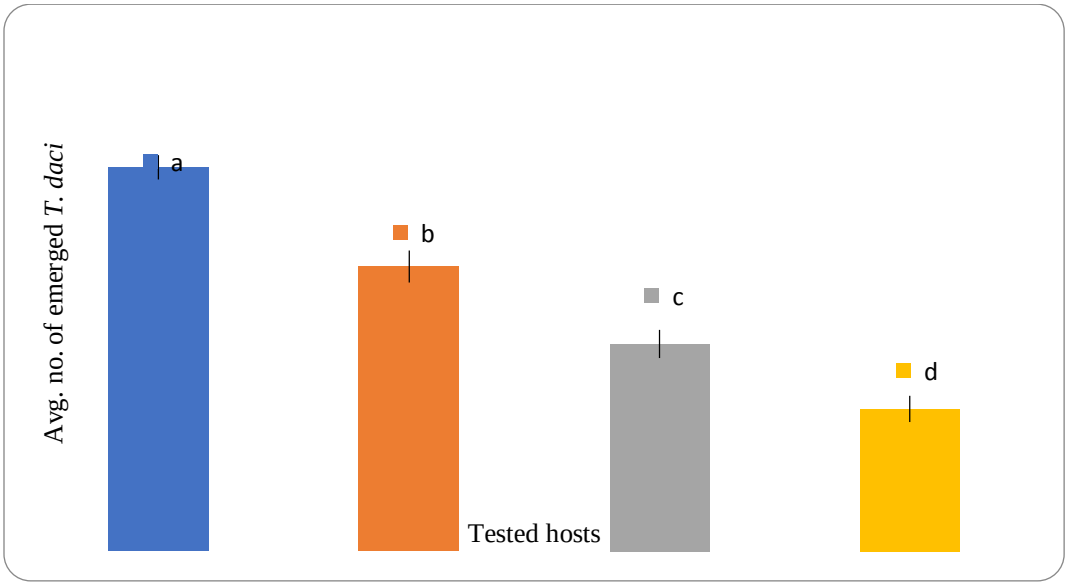

Fig. 2. Mean number of *T.chilonis*emerged from various hosts under laboratory conditions.

Effect of various hosts on unemerged pupae

The unemerged parasitoids from various hosts were statically fluctuated (P < 0.05) Fig. 3. Results revealed that maximum number of un-emerged *T.chilonis*(10.50±2.11) was recorded in *C. vesuviana* followed by *B. cucurbitae* and *B. dorsalis*, (8.30±1.17 and 6.10±1.02), respectively. However, least numbers of un-emerged adults were recorded in *B. zonata* (3.06±0.09).

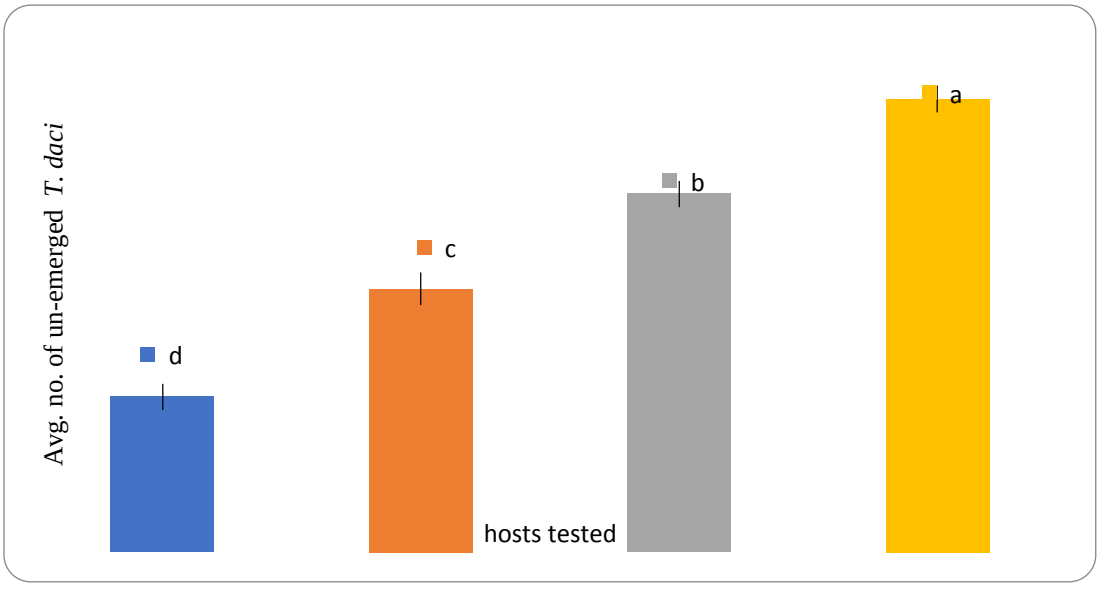

Fig. 3. Mean number of un-emerged *T.chilonis*from various hosts under laboratory conditions.

Effect of various hosts on emergence of males *T.chilonis*

There were significantly (P < 0.05) differences on the emergene of the male *T.chilonis*from various hosts as shown in Fig. 4.

Results revealed that maximum male emeregence was recorded in hosts of *B. zonata* (3.70 ± 0.05) followed by *B. dorsalis* (2.90±1.09) and lowest number of males was observed in *B. cucrbitae* and *C. vesuviana* (2.0±0.27 and 1.0±0.21), respectively.

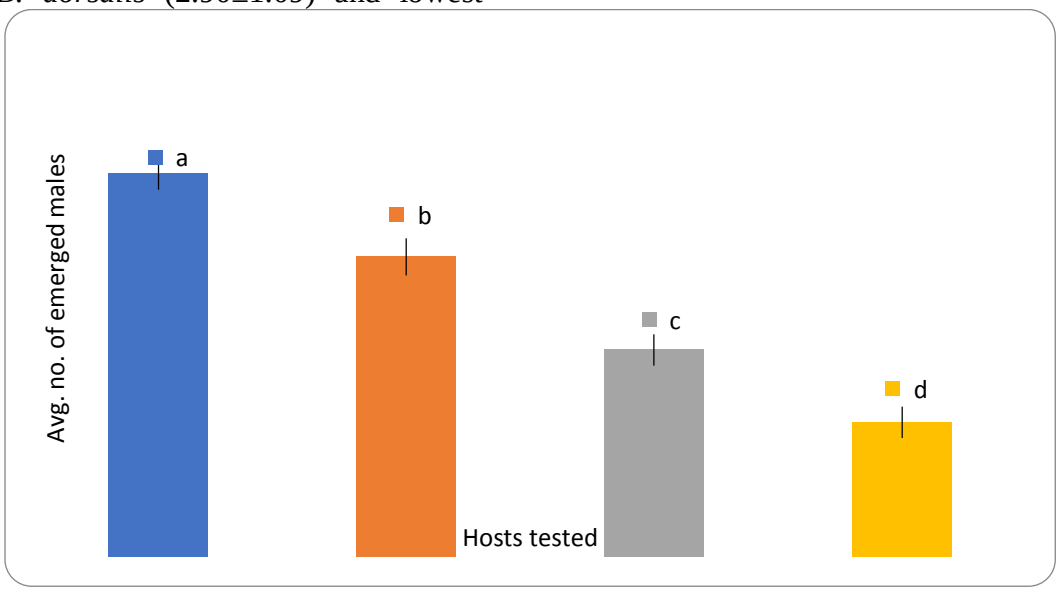

Fig. 4. Mean number of males *T.chilonis*emerged from various hosts under laboratory conditions

Effect of various hosts on female *T.chilonis*emergence

There were no significantly ($P < 0.05$) differences on the emergence of the females' *T.chilonis*from various hosts as shown in Fig. 5. Results revealed that maximum female emeregence was recorded in hosts of *B. zonata* (5.20± 1.45) followed by *B. dorsalis*, *B. cucurbitae* and *C. vesuviana* (3.70±0.90, 2.90±1.05 and 2.00±0.87), respectively.

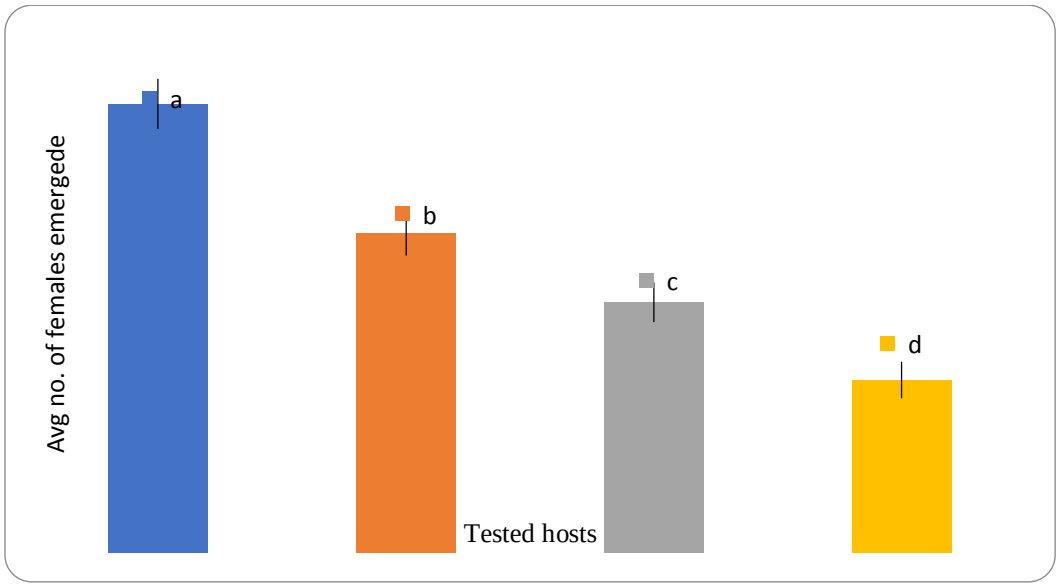

Fig. 5. Mean number of females *T.chilonis*emerged from various hosts under laboratory conditions.

Effect of various hosts on adult longevity *T.chilonis*emergence

Adult longevity of *T.chilonis*were significantly affected (P < 0.05) by the hosts (Fig. 6 and 7) maximum female longevity (9. 30 ± 2.05 days) was recorded when the *T.daci* were provided with *B. zonata*; the minimum (7.90 ± 2.51days) when fed on *C. vesuviana*. However, maximum male longevity (7.70± 1.89 days) was recorded when the *T.daci* were provided with *B. zonata*; the minimum (6.00 ± 1.09 days) when fed on *C. vesuviana*.

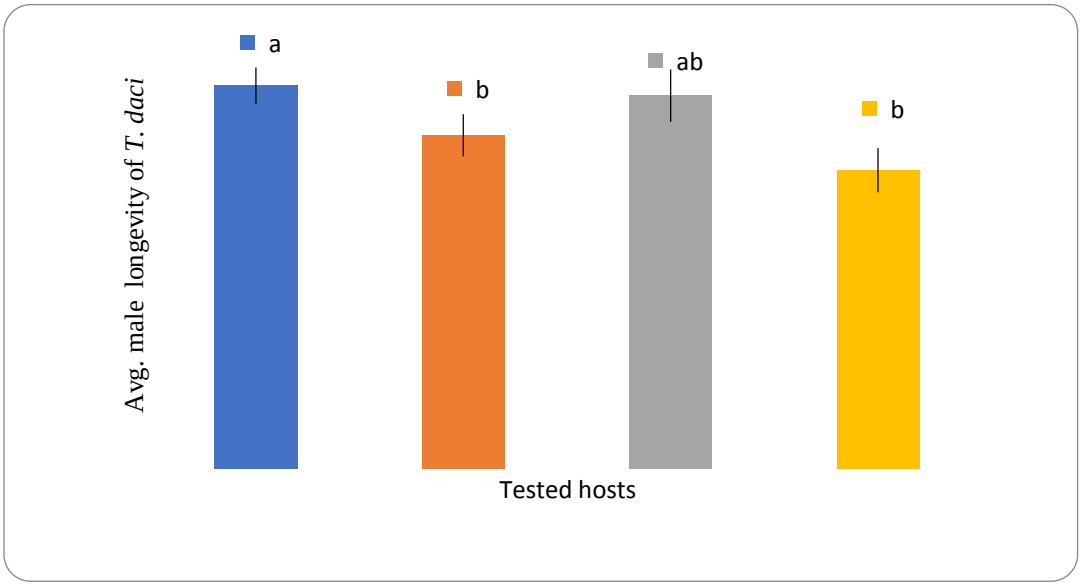

Fig. 6. Mean longevity of males *T.chilonis*emerged from various hosts under laboratory conditions.

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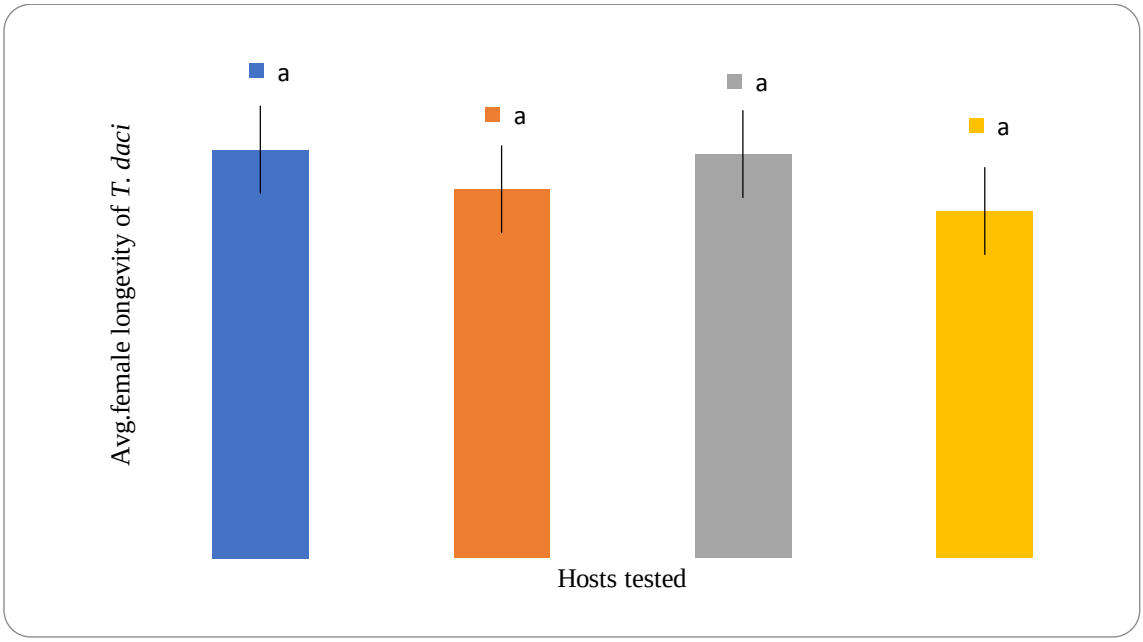

Fig. 7. Mean longevity of female *T.chilonis*emerged from various hosts under laboratory conditions.

Discussion:

Fruit flies in the family Tephritidae are high profile insects among commercial fruit and vegetable growers, marketing exporters, government regulatory agencies, and the scientific community. Locally, producers face huge losses without some management scheme to control fruit fly populations (McPheron & Steck, 1996). Fruit flies attack fruits and vegetables and not only reduce their yield but also affect the quality. The host fruits and vegetables attacked by fruitflies like *Bactrocera zonata* and *Bactrocera dorsalis* include guava, plum, peach, apricot, loquat, bitter gourd, citrus, mango, sponge gourd and pear.

The results of the present study reveals that the highest number of parsitized pupae, total number of emerged parasitoid, total number of male parasitoid and total number of female parasitoid pupae were recorded by fruit fly *Bactrocera zonata* (11.40±00.96, 8.90±0.87, 3.70±0.05 and 5.20±1.45 percentage), respectively, which were reduced to 8.90±1.19, 6.60±1.17, 2.90±1.09 and 3.70±0.9 percentage, respectively under *Bactrocera dorsalis* and further reduced to 6.70±0.94, 4.80±1.30, 2.00±0.27 and 2.09±1.05 percentage under *Bactrocera cucurbitae*, respectively. While the lowest number of unemerged and parsitzed pupae, total number of emerged parasitoid, total number of male parasitoid and total number of female parasitoid pupae were recorded from fruitfly *Carpomya vesuviana* (4.60±0.96, 3.30±0.94, 1.0±0.2 and 2.00±0.87 percentage), respectively under laboratory condition. Adult Longevity of *T.chilonis*maximum female longevity (9. 30 ± 2.05 days) was recorded when the *T.chilonis*were provided with *B. zonata*; the minimum (7.90 ± 2.51days) when fed on *C. vesuviana*. However, maximum male longevity (7.70± 1.89 days) was recorded when the *T.chilonis*were provided with *B. zonata*; the minimum (6.00 ± 1.09 days) when fed on *C. vesuviana*.

The similar results are supported by Gupta *et al.* (1990) studied seasonal fluctuation of *Dacus zonatus* (*Bactrocera zonatus*) and *D. dorsalis* (*B. dorsalis*). They caught more *B. zonatus* adults. Liu and Chen (1992) reported that protein hydrolysate against *B. dorsalis* was the most effective bait attracting 45.4% of adult females and 35.6% of males. Cohen and Yuval (1995) reported that the fruit flies have been the subject of experimentation for many years. High fruit fly populations and the abundance of fruits throughout the year contribute to the status of fruitflies as the major pest of cultivated fruits. Allwood and Drew (1996) reported that the seasonal activity of *Dipterophagus daci* was dependent on the availability of its host and rainfall. They further reported that the population increased with the onset of higher temperatures and moisture level. Mahmood *et al.* (1996) reported that fruit flies of economic importance are *Bactrocera dorsalis, B. zonata*, *B. cucurbitae*, *Dacus ciliatus* and *Myiopardalis pardalina*. Pablo Montoya *et al.* (2000) recorded highly significant differences in percentage parasitism were found in release and control zones in backyard orchards. Ovruski *et al*. (2000) addressed the need for much more intensive research on the bioecology of native fruit fly parasitoids. Mohsin *et al*. (2003) recorded fecundity was 135 offspring/ female and mean parasitoids /host puparium was 21.1. It was also developed in the laboratory on the Mediterranean fruitfly, *Ceratitis capitata* (Wiedemann), with an average life span of 23 days in both host species. Nikos *et al*. (2003) reported that the high parasitism rates were recorded in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in pupae derived from field infested figs, on the Greek island of Chios in 1999 and 2000. Adult parasitoids were identified as *Aganaspis daci* (Weld) (Hymenoptera: Eucoilidae), previously known as *Trybliographa daci* (Weld). Approximately 45% of *C. capitata* pupae yielded adult parasitoid in both years and the total mortality of pupae due to the parasitoid was 62-65%. Development of male *A. daci* at 25 °C, reared on 3rd

instar larvae of C. capitata, was shorter than that of the female (≈34 and 37 days respectively). Average adult male longevity was 4-5 days longer than female (16-17 to 11-12 days, respectively) and almost identical in wild and F1 parasitoids of both sexes. Babu and Shashidar (2003) reported that *Bactrocera dorsalis* was the dominant fruit fly among the 4 species (including *B. zonata*, *B. correctus* and *B. cucurbitae*) captured in methyl eugenol traps established in mango orchards. They recorded maximum infestation reached 30% (0.75 larvae per fruit). *B. zonata* and *B. correctus* populations had significant correlation with temperature, while *B. dorsalis* and *B. cucurbitae* populations had non-significant correlation with weather parameters (including relative humidity and rainfall). Shahata *et al.* (2008) indicated that most of *B. zonata* adults emerged between 6 a.m and 12 at noon but the maximum emergence took place between 9 a.m and 11 a.m. Andleeb *et al*. (2010) determined the importance of *Aganaspis daci* (*Trybliographa daci*) and *Diachaishmimorpha longicaudata* parasitoids in the use of fruit fly control, biology of *Aganaspis daci* was studied under controlled temperature and humidity conditions. *A. daci* was found to be more dominant and easy to use as a biological control agent than the *D. longicaudata.* Sabater *et al*. (2012) discussed the several specimens of *A. daci* were recovered from medfly larva collected from fig and citrus fruits in summer 2009. Analysis of COI and ITS sequences confirmed the taxonomical identification of Spanish specimens as belonging to the *Aganaspis daci* species by comparison to individuals from Greece, Israel, Hawaii and Egypt. Close species *Aganaspis pellenaroi* (Brethes) and *Ganaspis xanthopoda* (Ashmead) were used as outgroup for the phylogenetic analysis. Since 2011 a year round surveillance was established with medfly infested sentinel apples as larval parasitoid attraction trap. *Aganaspis daci* was first detected late in June. Laboratory experiments showed that parasitism rate is modulated by host density, and fertility is greater than *D. longicaudata*. Low temperatures seem to induce quiescence, and increase mortality rates at immature stages. Implications for mass rearing of *A. daci,* as well as the implementation of biological control based on this parasitoid were discusse

Conclusion

It was concluded from the studiesthat *B. zonata* was the most suitable host for the economical and efficient mass production of *T.chilonis*in terms of preimaginal growth, pupal measurement, parasitization and adult emergence. Moreover, bigger adults were recorded when *T.chilonis*was provided with the larvae of *B. zonata* as a host.

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