INTRODUCTION

Mango (Mangifera indica L.) is infested with 250 species of plant-feeding arthropods throughout the world. About 26 of these produce galls on various organs of mango tree (Peña and Mohyuddin, 1997; Raman et al., 2009; Gagne and Medina, 2004). Most of the mango gall inducing species belong to genus Procontarinia (Cecidomyiidae: Diptera) (Boucek, 1986).

Procontarinia matteiana Kieffer & Cecconi is a common gall midge on mango in India, Guadeloupe, Brazil, and West Indies, Kenya, South Africa, Java, Indonesia and Iran (De Villiers, 1998; Askari & Radjabi, 2003). In India, it attacks mango throughout the year prominently during vegetative and fruit maturity period (September and April) of the crop (Kaushik et al., 2012). P. matteiana was reported an economic pest during 1980s in Indian Gujarat, as it damaged 25.80 to 47.70% leaves of 3 varieties (Alphonso, Kesar and Rajapuri) of mango in 17 places (Jhala et al., 1987). This pest can cause serious damage in the absence of natural enemies (Austin, 1984). In Oman, P. matteiana caused considerable damage to mango plantations which necessitated a search for natural enemies to control it (Austin, 1984).

In surveys on mango gall midges made in Pakistan, P. matteiana was recorded one of the most serious pests of the gall midge complex. The results of studies on its phenology, distribution, biology, population trends and natural enemies at Rahim Yar Khan are reported here.

MATERIALS AND METHODS

Survey sites
Mango orchards were surveyed for gall midges and their natural enemies in 2007-09 at different localities in Punjab, Tranda Sway Khan (Rahim Yar Khan: 28.3°N, 65.23°E), Regional Agricultural Research Institute (Bahawalpur: 29.59° N, 73.19° E), orchard near Bahaudin Zakaria University at Bosan Road, (Multan: 31.32° N, 71.4°E) and Thokar Niaz Beg (Lahore: 32.2° N, 74.2° E).
Biology, phenology and population development of P. matteiana and its parasitoids

The mango orchard where regular observations of population development of P. matteiana and its parasitoids were conducted is situated at Tranda Sway Khan, a small town near Rahim Yar Khan. It consisted of about 302 mango trees of different varieties including Chounsa (222), Sindhri (20), Fajri (15), Dosehri (21), Anwar Ratol (4), Late Chounsa (1), Sarooli (6), Surrakh Sarooli (4), Tota Pari (2), Langra (2), Lahotia (2) and Desi (3). Most of the trees were about 30-40 years old while a few were 5-15 years.

The studies on the biology, phenology and population trends of P. matteiana and its parasitoids were conducted on commercially important variety of mango, i.e. Chaunsa. For this purpose 50 mango leaves, 10 each from five twigs from top of the branch at about 1.5 m height above ground were taken at fortnightly intervals. The twigs carrying these leaves were kept in jars with their lower ends dipped in water. Leaves infested with the gall midges were examined for numbers of P. matteiana and its parasitoids.

To study the biology of P. matteiana, mango seedlings with newly developed leaves were placed in jars in the laboratory together with field collected infested leaves for 7 days so that the adults emerging from them could lay eggs on the new leaves. The leaves having signs of oviposition were tagged and the seedlings were shifted to field cages (Fig. 1A-C). Tagged leaves were examined daily for stage of development of P. matteiana (egg to adult).

Management

Mass rearing of parasitoids

Locally available parasitoids of P. matteiana were reared in the laboratory and field and released in the experimental orchard. Their effect on the population of P. matteiana was calculated in terms of degree of parasitism.

To rear parasitoids in the laboratory, mango twigs infested with P. matteiana were dipped in small jars filled with water. The small jars were placed in large plastic jars covered with muslin cloth to collect the parasitoids emerging from P. matteiana galls. Adult parasitoids emerging from galls were collected with aspirator in glass vials. The mouths of glass vials were covered with muslin cloth secured with rubber band for ventilation. These vials were then transported to the experimental orchards for releasing on weekly basis.

In the field the culture of P. matteiana and its parasitoids was maintained in cloth tunnels (3X2X2 m) secured by bamboo sticks in the orchard. More than 7000 mango seeds were grown in the field on small beds after the mango picking season closed (August, 2008). When seedlings reached a stage of 3-5 leaves, they were taken out with earthen balls by digging the soil. Before digging, nursery beds were irrigated for optimum softening of the soil. These seedlings were then shifted to small plastic bags and placed under mango trees for few days for their establishment in the plastic bags in February – March, 2009. Some mango seedlings were also infested with P. matteiana. Established mango seedlings were finally transferred to above described cloth tunnels (@500/tunnel) (Fig. 1D, E). Some mango seedlings with galls on the leaves, infested dry twigs and fallen leaves with galls of P. matteiana were also placed in the tunnel to rear gall midges adults from them. During the experiment, dead plants even if infested leaves were present were removed and replaced with live plants. The adults of P. matteiana emerging from galls of infested seedlings, twigs and fallen leaves oviposited on newly developed leaves of seedlings kept in the tunnels. The parasitoids emerging from infested plants also oviposited on galls of P. matteiana. Parasitoids emerging from the twigs collected from infested trees that were reared in the in the laboratory were also released into tunnels to enhance oviposition rate on the galls of P. matteiana. The mango seedlings treated in this way had mostly parasitized galls. They were placed under preselected mango trees (700 seedlings/tree) in an experimental orchard on first appearance of midges in 2009-10 (Fig. 1F, G). These trees had been kept free from insecticides since 2008.

Data on number of adults of P. matteiana and its parasitoids/50 infested leaves was recorded at fortnightly intervals from preselected trees before after the release of parasitoids.
Fig. 1. Steps involved in studying biology and mass rearing of gall midge and parasitoids, *S. temporale* and *C. pulcherrimus*. Mango seedlings with newly emerged leaves and old leaves with galls of midge in jars (A), leaves with signs of oviposition of *P. matteiana* (B), seedlings shifted to field cages (C), and then to the cloth tunnels (D,E), mango seedling carrying *P. matteiana* galls mostly parasitized (F), seedlings with parasitized galls under mango trees in experimental orchard for release of parasitoids (G). More details in materials and methods section.

RESULTS

Procontarinia matteiana Kieffer & Cecconi

This species is for the first time reported from Pakistan. The specimens were identified by experts from Natural History Museum (NHM), London, UK. It was recorded from all areas surveyed in Punjab. Gall midges caused solitary or grouped galls on the upper and lower surfaces of the leaves. Thickness of solitary galls ranged from 3-3.25 mm and diameter 4.4 mm. In case of severe attack of this species, leaves became curled and ultimately dried (Fig. 2).

Phenology

This species is multivoltine. All stages, egg, larva, pupa and adult were recorded from March to November. Only the larval and pupal stages were detected in the winter months (December onward to February) in galls.
Biology

On hatching larvae entered leaves and started forming galls. At the beginning of gall development it was light green, increased in size and gradually became hard and concave at oviposition site. In July 08 at Rahim Yar Khan they completed development from egg to adult in 40 to 45 (mean 42.5±3.6) days. The abdomen of males is brown and females is light green (Fig. 3).

Population trends

Observations on population trends ran from April 08 at Rahim Yar Khan. In 2008 it was most abundant (N = 500) in April (Fig. 5) and from then onwards its population remained low. The population again started increasing in July, reached its peak (N = 125) in second week of September; numbers decreased in October and November and it was not recorded in winter months December 08 and in January and February 09. Though the population was very low compared with 2008, its maximum numbers were recorded in March (N = 80). The numbers decreased in April and remained very low afterward and could not increase its population up to September when maximum numbers (N = 73) were observed. In 2010 its numbers started increasing in February and reached at peak in March (N=150). In 2009-2010 the
population of *P. matteiana* remained low because of high numbers of the two parasitoids *Closterocerus pulcherrimus* and *Synopeas temporale*.

**Parasitoids**

*Synopeas temporale* Austin

This is known from India on *P. matteiana*. For Pakistan it is a new record. The specimens were identified by experts from Natural History Museum (NHM), London, UK. This parasitoid (Fig. 4) was reared from the galls of *P. matteiana*.

**Biology, phenology and population trends:**

These parasitoids completed egg to adult development within host gall. After completing development adults emerged from the galls. Its phenology is well synchronized with its host *P. matteiana*. It was a dominant parasitoid of *P. matteiana*, apparently breeding throughout the year. The three years (2008-10) study indicates that its population was high in March/April and
September/October (Fig. 5). Its breeding rate slowed down in winter 8-months (November – February) when it was reared in small numbers.

Closterocerus pulcherrimus (Kerrich)

Its known host is P. matteiana and is distributed in India, Pakistan, Sri Lanka, UAE, and Afro tropical. The specimens were identified by experts from Natural History Museum (NHM), London, UK. This parasitoid was reared regularly from the galls of P. matteiana. (Fig. 4)

Biological, Phenology and population trends: This parasitoid completed its development within the host gall and after completion of development only adults emerged from the galls. This parasitoid was reared from galls of the host P. matteiana from middle of March to November (Fig 5). It was not reared in winter months (December – February). Though this parasitoid was reared together with S. temporale its numbers remained lower than S. temporale.

Management

The impact of parasitoids was determined in terms of parasitism in experimental orchard at Rahim Yar Khan where regular observations on population trends of P. matteiana and its parasitoids were being followed since inception of the studies. For this purpose, more than 7000 mango seedlings with gall midges, most having been parasitized were placed under the marked mango trees in 2009-2010 kept free from pesticides since 2008.

The population of P. matteiana on the trees where these mango seedlings were placed decreased about three fold compared with the population of these gall midges in the year 2008. Parasitism success of C. pulcherrimus and S. temporale increased and the population of host P. matteiana decreased. In 2008, before the application of parasitoids the population of P. matteiana was 81.35% which decreased to 27.25% in 2009-10 after regular releases of parasitoids. Similarly the population C. pulcherrimus and S. temporale were 3.72 and 14.91 %, respectively in 2008. Their numbers increased to 17.69 and 55.05% in 2009-10 after the implementation of biological control (Fig. 5).

DISCUSSION

During the present study at Rahim Yar Khan, annually two generations were observed in the population of P. matteiana; first in March/April and second in September/October. This result coincides with the observation of Botha and Kotzé (1987). They found two generations of P. matteiana in South-Africa; first appeared in February-March and the other in October-November. This result is also similar to observations of Kaushik et al., (2012) who observed two generations in India; first in April and second in September. However, our observation differs from Askari and Radjabi (2003). They reported three overlapping generations in Syahoo and four in Minab Iran. In the same way, Gupta (1952) also reported three generations per year in India.

The result of this research indicates that P. matteiana completes its life cycle within galls. The same observations have also been reported by Botha and Kotzé (1987) and Askari and Radjabi (2005). Findings of this present study show that this pest completes its life cycle in 42.5±3.6 days. This corresponds the results of Askari and Radjabi (2003) who demonstrated that this pest completes its development in 46.594± 0.933 days in Iran.

Present work also reveals that the phenologies of two parasitoids S. temporale and C. pulcherrimus are well synchronized with their host and they are good capable of regulating the population of the pest. Some initial attempts of their mass rearing showed positive impacts in reducing the population of P. matteiana from 81.35 to 27.25%. This finding coincides with observations of Annecke and Moran (1982). They reported that parasitoids attack the mango gall midges so heavily that only parasitoids emerge from galls. This creates the incorrect impression that they are not parasitoids but gall inducing wasps. This phenomenon is probably not happening in the regions where the mango gall midges have been introduced accidentally and became pests of economic significance in the absence of natural enemies.

In conclusion, it seems P. matteiana is the most common and widely distributed gall midge pest in all mango growing areas of Punjab, Pakistan. It forms solitary or grouped galls on the upper and
lower surfaces of the leaves. It is multivoltine and remains active almost throughout the year with highest populations in April and September. It completes larval and pupal stages in plant tissues, adults emerge from the galls through small holes and females oviposit on young leaves. It completes its whole life cycle in 42.5±3.6 days. However, there is need for more detailed research work on its biology.

Two parasitoids S. temporale and C. pulcherrimus are new records from Pakistan. Both parasitoids attack the galls of P. matteiana and their phenologies are well synchronized with their host. Since they were well adapted to the local environment, they were selected for mass rearing. Preliminary efforts on mass rearing and introduction of these parasitoids indicated very positive impact on controlling the population of P. matteiana. However, much needs to be done to improve and encourage the use of these mass rearing techniques for application on a large scale to achieve long term control of pest. We also recommend more research work is needed on biology of these parasitoids.

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