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# Occurrence, Monitoring Techniques and Management of *Dasineura amaramanjarae* Grover (Diptera: Cecidomyiidae) in Punjab, Pakistan

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**Abstract.-** Mango, *Mangifera indica* L. ranks second in the fruit industry of Pakistan. Gall midges complex has been recently reported as one of the major pests from all mango growing areas of Pakistan. *Dasineura amaramanjarae* Grover is significant mango gall midge pest as its larvae feed in flower buds and may cause 100% reduction in yield. We recorded this pest from all mango growing areas of the Punjab including Rahim Yar Khan, Bahawalpur and Multan. Higher infestation was noted on mango trees in commercial orchards as compared to the trees scattered in small patches in farmer fields grown for personal consumption. Yellow sticky traps were more effective than green, blue, and colorless traps for monitoring of adults. During two consecutive growing seasons the adults of the pest were observed from February to April at Rahim Yar Khan. The use of neem seed kernel extract (NSKE) with integration of racking of soil under the mango tree was the second best option after bifenthrin for the control of the pest followed by spray of NSKE on mango canopy on the soil, and the racking of soil.

**Key words:** *Dasineura amaramanjarae* Grover, gall midges complex, *Mangifera indica*, neem seed extract.

## INTRODUCTION

Mango, *Mangifera indica* L. (Anacardiaceae) has been in cultivation in tropics and subtropics for several thousand years (Mukherjee, 1953, 1972; Purseglove, 1972). It is grown over 90 countries in the world and ranks fifth among major fruit crops. Asia accounts for approximately 77.0% of global mango production whereas the USA and Africa account for approximately 13.0% and 9.0%, respectively. Pakistan is also one of leading mango-producing and exporting countries. During 2003–2005, it contributed 4.5% and 6.9% to world production and export trade, respectively. This country is the major supplier of mango to the West Asian market (Litz, 2009).

In Pakistan, the potential yield per hectare of mango is 21.6 tons as compared to its average yield of 9.96 tons. This shows a wide difference that is attributed to multifarious factors like insect pests, diseases, weather hazards, defective marketing system and poor post and pre harvest practices (Srivastava, 1998; Lodhi *et al.*, 2006).

About 250 insect pests of mango have been recorded in the world. Among these, gall midges, caterpillars, leafhoppers, thrips, and mites are the most important pests attacking mango (Peña and Mohyuddin, 1997; Peña *et al.*, 1998). Gall midges have recently been emerged as a pest of mangoes in Pakistan. They have been recorded since last decade in all mango growing areas of Pakistan particularly in Multan, Bahawlpur, Lodhran, Rahim Yar Khan, Lahore and Faisalabad (Ahmed *et al.*, 2005; Rehman *et al.*, 2013). Researchers emphasized that this group of pest was needed to determine its status in the Punjab province.

Previous researchers from India reported that *Dasineura amaramanjarae* Grover **(**Diptera: Cecidomyiidae) was recorded as a significant gall midge pest of mango which causes damage due to direct feeding of its larvae on flower buds. In the case of severe infestation, the reduction in yield can reach 100%. Usually, 3-4 larvae feed in a single bud but their number can increase up to 6-8 or even 10-12 where several females have oviposited (Prasad and Grover, 1966).

The female of *D. amaramanjarae* has been reported to lay eggs near the stamens. A single female can lay 40-50 eggs, up to six eggs per bud. They are hatched in 30-36 hours, depending upon

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the temperature and humidity. There are four larval instars and the most the damage is caused by the second and third ones. The larvae at final stage drop to the soil for pupation or diapause (Grover and Prasad, 1966).

In Pakistan, flowering on mango starts in February and fruits become mature in June/July depending upon type of cultivars and mango growing area (Iqbal *et al.*, 2012). Recent research reports that the appearance of the adults of *D. amaramanjarae* has a close synchronization with initiation of flowering on mango trees in Punjab, Pakistan. They emerged from the soil after completion of pupation/diapause and cause damage from February to April *i.e.* during the availability of flowers. These have not been recorded on the mango orchards from May to January when mango flowers are not available (Rehman *et al.*, 2013a).

Light trap, sticky traps of different colors and plastic sheet method have been employed for the detection of gall midges in world (Prasad, 1968; Kashyap, 1986; Sharma and Franzmann, 2001; Plažanin *et al.*, 2012). However, there is scarcity of literature on the detection methods and the pest status of *D. amaramanjarae* from mango growing areas of Pakistan. In present study, we report its occurrence in mango growing areas/orchards, monitoring techniques and management in Southern Punjab Pakistan.

## MATERIALS AND METHODS

*Severity of infestation of* D. amaramanjarae

Severity of *D. amaramanjarae* infestation at major mango growing areas was studied in commercial mango orchards having no intercrops planted in a geometry and keeping uniform distance from tree to tree at three locations *i.e.* Taranda Saway Khan, Rahim Yar Khan (District Rahim Yar Khan: 28.3°N, 65.23°E), Regional Agricultural Research Institute, Bahawalpur (District Bahawalpur: 29.59° N, 73.19° E), and Bosan Road, Multan (District Multan: 31.32° N, 71.4°E) during 2010 and 2011. These orchards consisted of different mango varieties like Chounsa, Tota Pari, Langra, Sindhri, Anwar Ratool, Dosehri, Surrkha Sarooli, Late Chounsa, Lahotia, and Dasi. Mango trees were 8-40 years old. Annually, they were given 5-14 irrigations and 1-2 ploughing and manuring.

In March during 2010 and 2011, four trees were selected randomly at each of location and 15 inflorescences were taken from each of four mango trees. Inflorescences where larva was found were considered infested. Small farmers also plant mango trees usually intercropped with wheat and cotton for producing fruits at small scale. Infestation of *D. amaramanjarae* was also recorded from five trees at three locations with similar methodologies mentioned earlier at same locations. Percent infestation was calculated from each locality with following formula.

$$\text{Percent infestation} = \frac{\text{Total Inflorescences-Healthy Inflorescences}}{\text{Total Inflorescences}} \text{ x100}$$

In both seasons 2010 and 2011, percent mean of adults with standard error (Mean±SE) on each locality was calculated. A comparison in infestation of *D. amaramanjarae* on mango trees among the commercial orchards at three localities, Rahim Yar Khan, Bahawalpur, and Multan was made by using analysis of variance (ANOVA). Same test was also used for comparison of pest infestation among the orchards grown by small farmers at three locations. Student *t*-test was used for comparing the population of pest between the commercial orchards and the orchards grown by small farmers.

*Relative effectiveness of different traps for monitoring of* D. amaramanjarae

Three sticky traps yellow, green, and blue measuring 10x10cm were tested for their efficacies to monitor the adults of *D. amaramanjarae* from January to April (for 120 days) in 2011 and 2012. We selected four mango trees and six traps of each color were hanged per tree in experimental orchard at Rahim Yar Khan. Each tree was considered as a replication. Six colorless sticky traps also tied as control on each four trees. Traps were hanged on the tree for 24 hour in a week and brought to laboratory in plastic bags. Adults of *D. amaramanjarae* on the traps were counted using magnifying lens. Mean numbers of adults with standard error (Mean±SE) on each trap was calculated and the efficacy of four

color traps was determined by making comparison with ANOVA. LSD test (95%) was used for separating the difference among various traps.

*Management of* D. amaramanjarae

During two mango inflorescence seasons in 2011 and 2012, trials on management of *D. amaramanjarae* were conducted at Rahim Yar Khan in randomized complete block design (RCBD) Water solution of neem seed kernel extract (NSKE) was prepared by grinding and mixing it (@10 g/100 ml) in distilled water for 3-4 h in a beaker. It was then filtered through a muslin cloth which was squeezed into the beaker. The suspension thus obtained was taken as 10.0% solution (Singh and Singh, 1998).

**Table I. - Treatments evaluated for the managing *D. amaramanjarae* at Rahim Yar Khan during 2011 and 2012.**

| | Treatments |
|---|---|
| $T_1$ | Spray of 10.0% NSKE on the soil under mango tree |
| $T_2$ | Spray of 10.0% NSKE on canopy of mango tree |
| $T_3$ | Spray of NSKE on mango tree canopy + racking (hoeing) of soil under the mango tree canopy |
| $T_4$ | Racking (hoeing) of soil under the mango tree canopy |
| $T_5$ | Talstar, 10 EC (Bifenthrin, an insecticide of pyrethroid group from FMC United group Lahore) sprayed by farmer with tractor mounted boom sprayer @ 125ml/250 L of water for one hectare |
| $T_6$ | Control |

We selected five mango trees (Chounsa variety) for each treatment and each tree was considered as replicate. There were thus total 30 trees for the experiment. These treatments are listed in Table I. During each year 2011 and 2012, hoeing and spraying of NSKE was done from December to April (for 151 days) at fortnightly intervals while bifenthrin was sprayed by farmer once in March. A plastic sheet (1x1m) was spread under the canopy of each replicate. Numbers of the larvae of *D. amaramanjarae* dropped on plastic sheets spread under all trees were counted daily from February to April (for 89 days) in 2011 and 2012. Mean numbers of the larvae per sheet (a tree) was calculated for each treatment and further analyzed with ANOVA. Differences among the treatments were separated by LSD test (95%).

## RESULTS

*Severity of infestation of* D. amaramanjarae

*D. amaramanjarae* Grover was recorded from all the areas surveyed in Punjab including Rahim Yar Khan, Bahawalpur, and Multan. It had earlier been reported from mango trees in India in 1960s and we reported it recently from Pakistan (Rehman *et al*., 2013a). Severity of infestation was determined in the terms of infestation on inflorescences of mango trees in commercial orchards and farmer fields at three localities. Higher density of the pest was observed on mango trees in commercial orchards than the scattered trees present in the fields of small farmers. In 2010, out of total inflorescences examined from the mango trees in commercial orchards, 85.3% were infested at Rahim Yar Khan, 77.2 and 81.5% infestation was recorded at Bahawalpur and Multan, respectively. Population of the pest among three localities was not significantly different ($F = 3.02$; $df = 2, 11$; $P < 0.12$). Mango trees planted in patches at small farmer fields received lower infestation of *D. amaramanjarae* at same locations *i.e.* 19.2, 9.0, and 11.0% at Rahim Yar Khan, Bahawalpur and Multan, respectively. There was no significant variation in damage of *D. amaramanjarae* on the trees at small farmer fields among three localities ($F=3.24$; $df=2, 11$; $P < 0.11$). In following year, 2011, commercial orchards at Rahim Yar Khan had highest infestation (50.5%) followed by Multan and Bahawalpur (46.2 and 38.0%, respectively). While on trees at farmer fields, lower infestation of the pest was observed *i.e.* 6.2, 5.5, and 4.5% at Rahim Yar Khan, Bahawalpur, and Multan, correspondingly. However, infestation did not vary significantly from site to site on mango trees in both commercial orchards and farmer fields ($F = 1.43$; $df = 2, 11$; $P < 0.05$) (Fig. 1).

Mango trees in commercial orchards were damaged more by *D. amaramanjarae* than isolated trees at farmer fields when compared with *t*-test. In 2010, the difference in infestation between trees in commercial orchards and in small farmer fields was significant at all places including Rahim Yar Khan ($t = 16.59$; $df = 3$; $P < 0.00$), Bahawalpur ($t = 10.83$; $df = 3$; $P<0.01$), Multan ($t = 14.52$; $df = 3$; $P<0.00$).

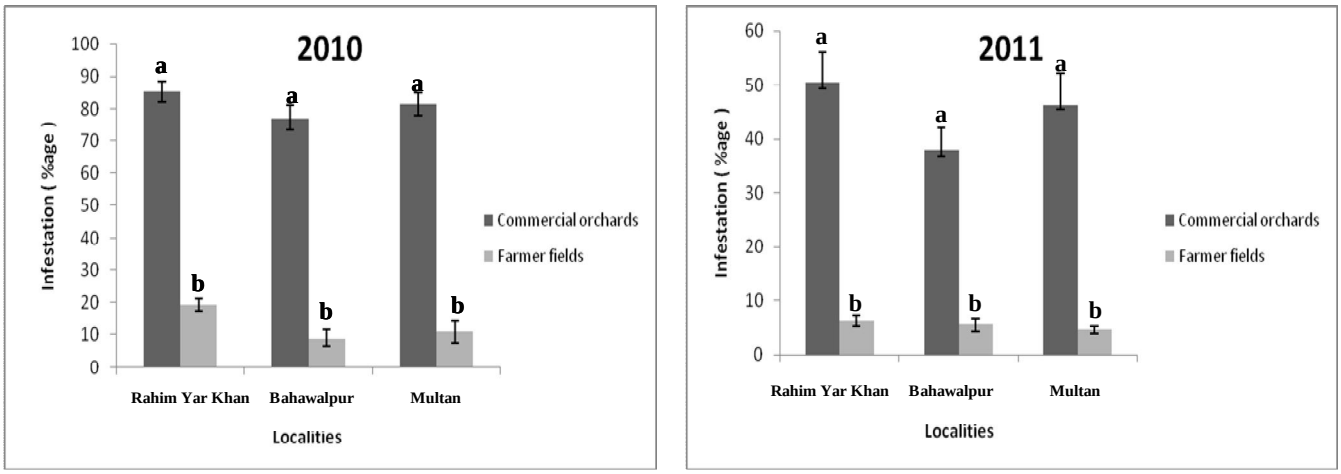


Fig. 1. Infestation (in % Mean±SD) of *D. amaramanjarae* on trees in commercial orchards and farmer fields of Rahim Yar Khan, Bahawalpur and Multan in 2010 (left) and 2011 (right). Bars topped with different letters are significantly different (LSD Test, $\alpha = 0.05$).

In the same way during the following season (2011) there was also significant difference between two types of mango trees at Rahim Yar Khan, ($t$ = 10.41; df = 3; P < 0.00), Bahawalpur ($t$ = 7.68; df = 3; P < 0.00), and Multan ($t$ = 7.74 df = 3; P < 0.00). (Fig. 1).

*Relative effectiveness of different traps for monitoring of* D. amaramanjarae

During 2011-12, the adults of *D. amaramanjarae* were caught on all traps at Rahim Yar Khan. The pest remained active at the site from February to April as observed in weekly captures on different color traps. Maximum numbers were observed in March (61.2%) followed by February (22.5%) and April (16.3%). Adults caught on yellow trap were 33.3% followed by green (27.5%), blue (24.7%), and colorless (14.5%) for the both years (percentages calculated from Figure 2). Figure 3 presents actual numbers of adults captured in different color traps however for comparison percentages calculated from the seasonal means for each color trap are described. In 2011, population of *D. amaramanjarae* was first observed in third week of February on all traps and continued up to first/second week of April. Percent means of adults recorded on yellow trap were 33.4% followed by green (28.4%), blue (26.7%), and colorless (10.5%). There was a significant difference among the mean numbers of adults on these four kinds traps (F = 5.05; df = 3, 15; P < 0.02). Numbers of adults on colorless trap was significantly less from all other three traps. However, no significant difference was observed in *D. amaramanjarae* population on the yellow, green, and blue traps in this season. (Fig. 3A). In next year 2012, adults were caught on all traps from second week of February to the third week of April. Maximum numbers were recorded in March (43.3%) followed by April (36.8%) and February (19.9%). Relatively higher numbers were attracted towards yellow (38.0%) followed by green (29.2%), blue (20.5%), and colorless (12.3%). There was a significant difference in attraction of *D. amaramanjarae* towards the four traps (F = 6.48; df = 3, 15; P < 0.01) (Figs. 2, 3).

*Management of* D. amaramanjarae

During 2011-12, neem seed kernel extract (NSKE), cultural practices and insecticide were applied on the canopy and the soil beneath the mango trees for the management of *D. amaramanjarae* at Rahim Yar Khan. Mean numbers of larvae plastic sheet$^{-1}$ were the relative estimate of damage by *D. amaramanjarae* on all treatments. In 2011, analysis of variance (ANOVA) detected a significant difference in the mean numbers of the larvae among the various treatments used for the management of the pest (F, 45.84; df, 5, 29; P<0.00).

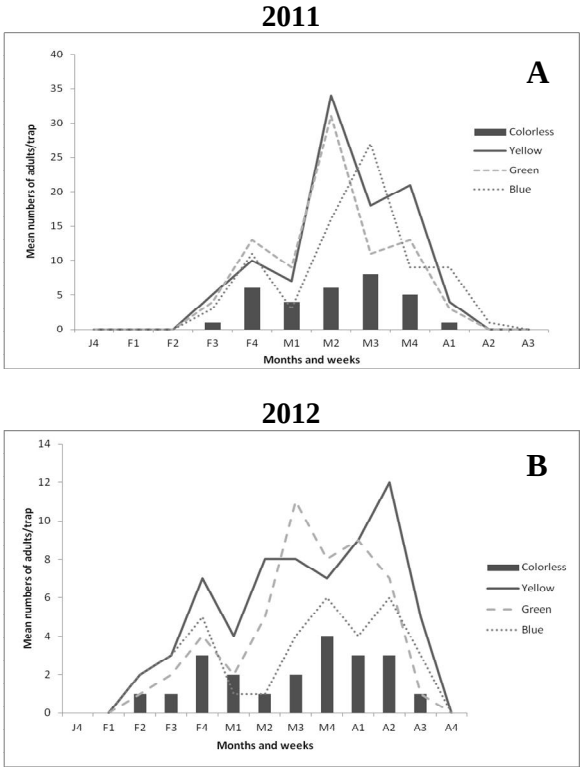


Fig. 2. Monitoring of *D. amaramanjarae* adults with traps of different colors at Rahim Yar khan in 2011 (A) and 2012 (B).

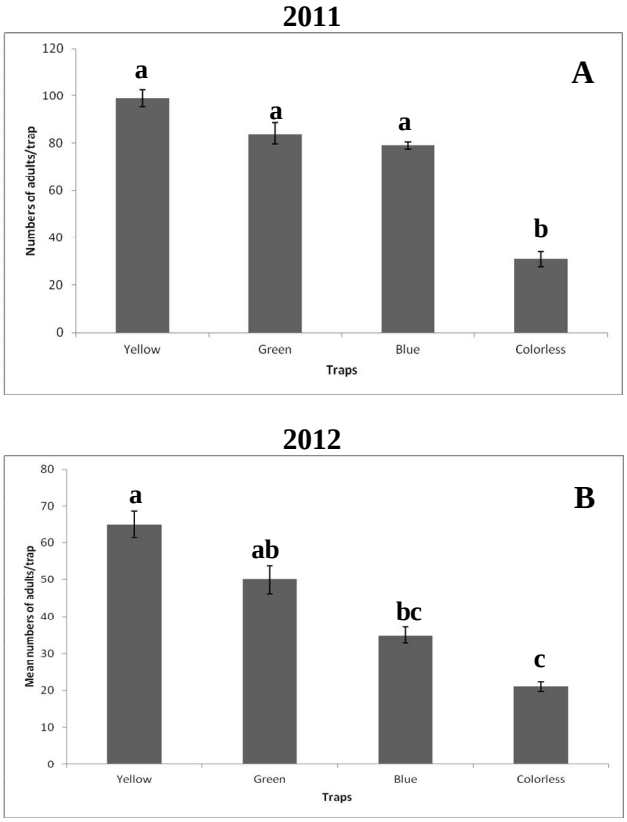


Fig. 3. Comparison of different color traps (Mean±SE) for monitoring of *D. amaramanjarae* adults at Rahim Yar khan in 2011 (A) and 2012 (B). Bars topped with different letters are significantly different (LSD Test, $\alpha = 0.05$).

To avoid presentation of relatively large figures of actual estimates of larvae (Fig. 4) we converted these estimates in to percentages of each treatment for sake of clarity to describe results here. The treatment of insecticide bifenthrin was superior over all other treatments in reducing *D. amaramanjarae* infestation to 11.6%. The next best was the combination of treatments NSKE spray on tree canopy and racking of soil which recorded 12.2% of infestation followed by NSKE spray on tree (13.74%), racking of soil alone (15.7%), and NSKE spray on soil (16.0%). Control treatment recorded highest percentage of *D. amaramanjarae* larvae (30.8%). Pair wise comparison with least significant difference test (LSD at $\alpha$ =0.05) showed that bifenthrin and NSKE spray on tree canopy+ racking had significantly better control of the pest than other treatments. Dunnett's Multiple Comparisons with control ($\alpha$=0.05) also indicated that numbers of larvae in all treatments were significantly lower than control. In 2012, the difference among the various treatments was also significant (F=65.74; df=5, 29; P < 0.00). Least infestation was observed on the treatment of bifenthrin (11.1%) followed by NSKE spray on tree canopy + racking (13.5%), NSKE spray on tree canopy (14.0%), racking of soil (15.4%), NSKE spray on soil (17.0%), and control (28.7%). LSD ($\alpha$=0.05) showed that mean larval population on four treatments, spray of bifenthrin on tree canopy, NSKE spray on tree canopy, NSKE spray on soil, control was significantly different.

## DISCUSSION

Studies on severity of infestation at different localities and planting pattern at Rahim Yar Khan, Bahawalpur, and Multan indicated that mango trees

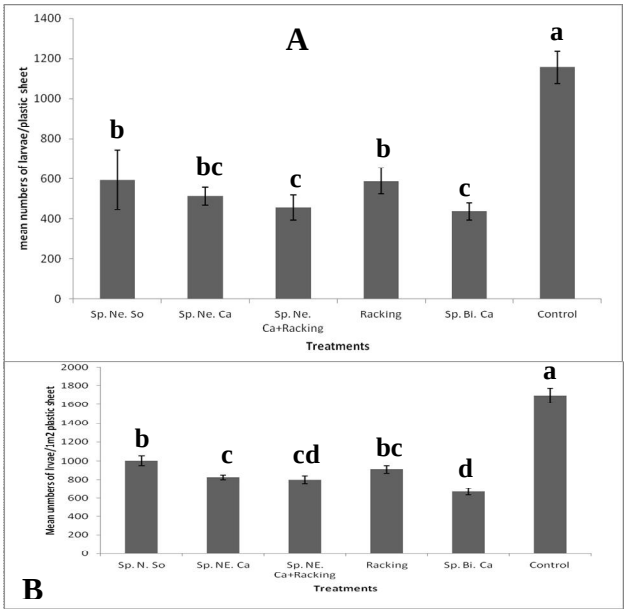


Fig. 4. Season long populations of *D. amaramanjarae* in different treatments (Mean±SE) at Rahim Yar Khan in 2011 (A), and 2012 (B). Sp. Ne. So, Spray of neem on soil; Sp. Ne. Ca, Spray of neem on canopy; Sp. Bi. Ca, Spray of bifenthrin on canopy. Bars topped with different letters are significantly different (LSD Test, $\alpha = 0.05$).

grown by small farmer in their fields received significantly lower infestation of *D. amaramanjarae* than commercial orchards on all sites during two years 2010 and 2011. The lower infestation may be due to the reason that small farmers grow mango trees usually intercropped with wheat and cotton and use more numbers of cultural practices (annually 10 ploughings and 5 hoeings) for seed bed preparation and weeding of these crops (Khalil and Amanullah, 2004). Another reason might be the availability of food source for midges which is obviously more abundant in commercial orchards. Farmers use only 1-2 hoeings in commercial orchards in a year. *D. amaramanjarae* pupates and hibernates in the soil under the mango tree and higher numbers of cultural practices may interrupt life cycle of the pest by breaking down its pupation and hibernating places under mango tree (Prasad, 1966; Grover and Prasad, 1966).

It was noted that maximum adult of *D. amaramanjarae* were caught on yellow trap followed by green, blue, and colorless. The phytophagous insects preferred yellow color more than darker colors such as blue and black (Meyerdirk *et al.,* 1979). Among the gall midges, sorghum gall midge, *Stenodiplosis sorghicola* females were attracted to yellow followed by green, red, and blue traps. They responded more quickly to yellow, followed by red, green, blue traps (Sharma and Franzmann, 2001). The use of yellow sticky traps has been documented for the monitoring of blueberry gall midge *Dasineura oxycoccana* Johnson (Plažanin *et al.,* 2012).

The results illustrated that bifenthrin was effective in controlling *D. amaramanjarae* and had lowest population over other treatments during two inflorescence seasons 2011-12. Bifenthrin has already been reported to be used for controlling insect pests of mango in Pakistan (Saifullah *et al.*, 2007). However, it has been reported to be toxic to some hymenopterans parasitoids (Prabhaker *et al.*, 2007). The results also revealed that the NSKE were the next best control option after insecticidal control. The neem based insecticides are considered as economical for controlling insect pests and safe for human beings and beneficial insects due to lesser residual toxicity (Caboni *et al.*, 2006; Hasan *et al.*, 1996). They act as systemic and as contact poisons and their effects are antifeedant, toxicological, repellent, sterility inducing or insect growth inhibiting. Furthermore, they are environmentally safe and have the potential to be adopted on commercial scale, together with other control measures in order to devise a low cost management strategy (Gahukar, 2000).

In conclusions, it appeared that *D. amaramanjarae* was found in all surveyed areas of Rahim Yar Khan, Bahawalpur and Multan. Significantly higher infestation was recorded on mango trees in commercial orchards as compared to the scattered trees in farmer crop fields. So a holistic integrated pest management approach recommended for the control of *D. amaramanjarae* in commercial orchards. For monitoring, yellow sticky traps were better in attraction of *D. amaramanjarae* than green, blue and colorless traps. As we noted that *D. amaramanjarae* was found active from February to April, therefore it is recommended to start monitoring of this pest with yellow traps from

February or with the initiation of flowering. However, there is need of further research to determine optimum numbers of yellow traps/tree or per unit area for monitoring purposes. We recommend detailed assessment of plant development scale (BBCH) of mango during active period of *D. amaramanjarae.*

The use of NSKE with integration of racking of soil under the mango tree was the second best option after bifenthrin for the control of the pest followed by spray of NSKE on mango canopy, NSKE spray on soil, and racking of soil. Initially, the use of NSKE with integration of racking of soil under the mango tree on a small scale (on five trees) proved very effective in controlling the pest population. It is also more eco-friendly than synthetic pyrethroid group and contact-gastrointestinal track insecticides. In the future, there is need to evaluate the impact of these techniques on large scale for sustainable control of the pest. It is also recommended to explore the natural enemies fauna of *D. amaramanjarae* in order to improve the of integrated pest management. Moreover, losses should be determined due to *D. amaramanjarae* and timing of application of pest management practices need to be explored along with development of economic threshold or action threshold levels.

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