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Laboratory evaluation of some insecticides against citrus mealybug *Planococcus citri*, (Homoptera: Pseudococcidae)

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Abstract
Citrus mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae), is a serious pest of citrus plants in Pakistan which cause considerable loss to citrus crop both in terms of quantity and quality. The present study was conducted to evaluate the toxic effect of different insecticides at different concentrations against *P. citri*. Leaf-dip bioassays were performed to assess the toxicity of a pyrethroid (Cypermethrin), an organophosphate plus pyrethroid (Polytrin-C), a neonicotinoid (Imidacloprid) and a synthetic IGR (Buprofezin), using 3rd instar female individuals of *P. citri*. Data regarding mortality of insects was recorded at 6, 12, 24 and 36 hrs post-treatment (HAT). Maximum mortality was observed in case of Polytrin-C (74.84%) followed by Buprofezin (65.15%) at 36 HAT, while Imidacloprid and cypermethrin were least effective. In case of different concentrations of each chemical, mean maximum mortality was observed at 4 ppm (67.61%) after 36 HAT. Study results suggest that Polytrin-C and Buprofezin are effective chemicals at 4 ppm against citrus mealybug. However, non-target effects of Polytrin-C, such as on biological control agents and other organisms should be determined.

Keywords: *Citrus sinensis*, Mealybugs, Pyrethroids, Synthetic insecticides, Toxicity.

Introduction
Mealybugs are one of the destructive insect pests and damage a large number of horticultural and agricultural crops such as citrus (Bodenheimer 1951) [9], mango (Ackonor 2002) [2], coffee (Staver *et al*. 2001) [24], and grapevine (Cid *et al*. 2006; Laflin and Parrella, 2004) [10, 16]. There have been about 175 genera belonging to 74 families of mealybugs described so far (Ben-Dov 2007) [8]. There is sexual dimorphism in mealybugs. Males are about 1 mm long crimson colored flying insects with two caudal filaments, while female mealybugs are about 3 mm long oval-shaped wingless individuals and exhibit simple metamorphosis. Females live for about 3 to 6 weeks while males are usually short-lived (Mahmood *et al*., 2014) [17].
Citrus mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae), is a major pest of citrus in Pakistan due to its polyphagous nature. It is a sporadic pest of citrus and attacks a wide variety of citrus cultivars (Kerns *et al*., 2012) [15]. It reduces the fruit quality by sucking at base of fruit-stalks, calyx and fruits, and causes drop of fruits due to weakening of stalk base. In addition, it causes the accumulation of honeydew and sooty mold (a fungus) growth on different parts of plants such as on fruits and leaves (Uygun *et al*., 2001) [25]. It can be successfully controlled by the application of natural enemies such as *Cryptolaemus montrouzieri* beetles (Singh, 2004; Al *et al*., 2010) [3, 23]. However, mealybugs have a cryptic nature and development of cushiony scales on the dorsal side of female mealybugs reduces the exposure and absorption of pesticides into its body, resulting into poor and ineffective control (Bartlett and Clancy 1972; Wysoki *et al*., 1981) [6, 26].
Keeping in view the destructive nature of citrus mealybugs, the main objective of this study was to evaluate the toxicity of insecticides belonging to different modes of actions at different concentrations against citrus mealybug.

Materials and Methods
The experiment was conducted in the Laboratory of Entomology, University College of Agriculture, University of Sargodha.

Insect Culture

Mealybug clusters were collected in early spring (March 2014) from the citrus (*Citrus reticulata* Blanco) nursery situated in the horticulture farm of the University College of Agriculture, University of Sargodha. They were brought to lab and were reared on 50-60 cm long potted plants of sour orange (*Citrus aurantium* L.) under 25 ± 3 °C and 60 ± 5% relative humidity.

Insecticides

Insecticides used in toxicity bioassays included a pyrethroid (Cypermethrin 10 EC), an organophosphate plus pyrethroid (Polytrin-C 440 EC; Profenofos+Cypermethrin), a neonicotinoid (Imidacloprid 70 WS) and an IGR (Buprofezin 25 WP). These insecticides were purchased from the local pesticide market of district Sargodha and each insecticide was tested at four different concentrations (i.e. 0.5, 1, 2 and 4 ppm).

Toxicity bioassay

Leaves of kinnow mandarin (*Citrus reticulate* Blanco) were collected from field and brought into laboratory of Entomology. Third instar female mealybug nymphs of homogenous age were collected from the laboratory culture. Leaf-dip bioassay was performed in which leaves were dipped into each insecticide solution for ten seconds and were air-dried for 3 min on filter paper. Then these treated leaves were transferred to Petri-dish on a moistened filter paper disc at the base. Total 15 mealybug nymphs were released in each petri-dish for each concentration of chemical. Each treatment was replicated four times. Data of mortality was recorded 6, 12, 24 and 36 hours after releasing insects. Percent mortality was calculated by using Abbot's formula (1925) i.e. $[(X - Y)/X] \times 100$, where X = % alive insects in control treatment; Y = % alive insects in insecticide treatment.

Data analysis

Apart from graphical presentation, data was subjected to analysis of variance (ANOVA) and means were compared using Tukey HSD all pair-wise comparison test.

Results

Percent mortality regarding the efficacy of different insecticides against citrus mealybug was recorded and subjected to statistical analysis (Table 1). The results showed that treatments and concentrations were highly significant at all-time intervals. At 36 HAT, treatments and concentrations showed a significant difference (F value = 272.18, P value = 0.0000 and F value = 63.96, P value = 0.0000), respectively. The interaction between treatments and concentrations showed non-significant result (F value = 1.62, P value 0.1358 and F value 0.70, P value = 0.7073) at 6 HAT and 12 HAT, respectively. The interaction was significant at 24 HAT (F value = 3.00, P value = 0.0064).

Mean comparison done through Tukey HSD all pair-wise comparison test showed that maximum mortality was observed in case of Polytrin C (46.13% and 74.84% at 24 HAT and 36 HAT, respectively). The second effective insecticide was Buprofezin which showed 44.14 and 65.15 percent mortality at 24 HAT and 36 HAT, respectively. The least significant result was observed in case of Imidacloprid (30.88%, 36.87%) at 24 HAT and 36 HAT, respectively (Fig. 1). In case of concentration of each chemical, the maximum mortality was observed at 4 ppm (53.32%, 67.61%) after 24 HAT and 36 HAT respectively. The percent mortality of *P. citri* was also significant at 2 ppm (42.85, 61.95%) after 24 HAT and 36 HAT respectively. The least significant results were observed in case of 0.5 ppm on which mealybug mortality was (27.27%, 49.91%) after 24 HAT and 36 HAT, respectively (Fig. 2).

Table 1: Analysis of variance for toxicity of different insecticides against 3rd instar female *P. citri*

		6 HAT		12 HAT		24 HAT		36 HAT	
Source	**DF**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Treatments(T)	3	50.67	0.00	30.02	0.00	60.68	0.00	272.18	0.00
Concentrations(C)	3	98.92	0.00	75.01	0.00	154.28	0.00	63.96	0.00
T X C	9	1.62	0.13	0.70	0.71	3.00	0.01	1.86	0.08
Error	48								
Total	63								

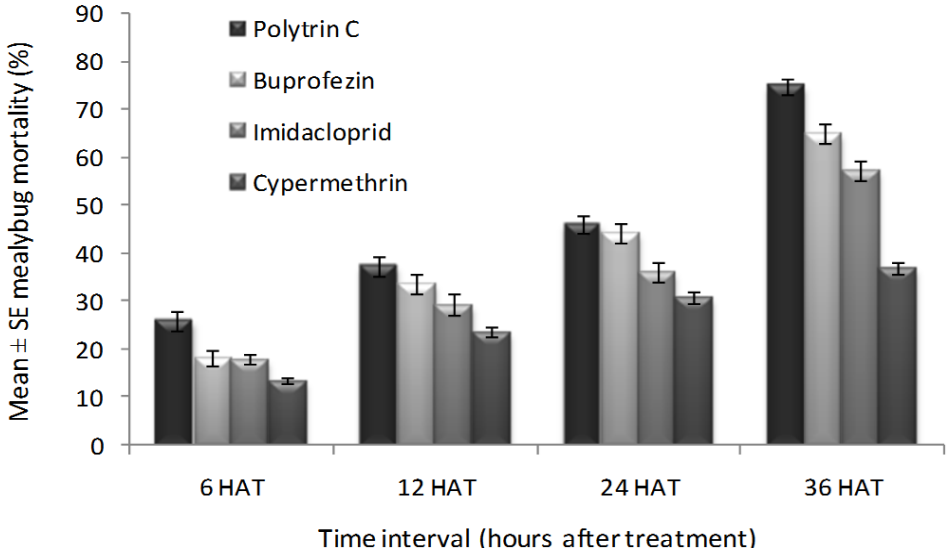

Fig 1: Mean toxicity (% mortality) of 3rd instar female *P. citri* against different insecticides

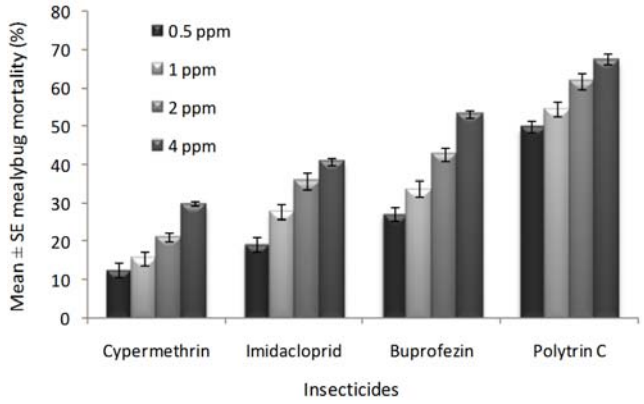

Fig 2: Mean toxicity (% mortality) of 3rd instar female *P. citri* at different concentrations of insecticides tested

Discussion

Citrus mealybug, *Planococcus citri*, is an emerging threat to citrus orchards in Pakistan, particularly in Sargodha district for the last decade (Anonymous, 1969: Fayyaz-ur-Rehman, 2009) [4, 11]. In case of severe infestation and no control, 100% fruit drop or 80% defoliation has been observed (Kerns *et al*. 2001; Arif *et al*. 2012) [5]. Consequently, citrus growers have been using different insecticides for mitigating these devastating pests with mixed control success. The present study was aimed to evaluate the comparative efficacy of different insecticides at different concentrations against citrus mealybugs in order to find out the most effective insecticide and its concentration to be suggested to farmers as chemical control option.

Results of this laboratory study showed that combination insecticide, Polytrin-C, is more promising against *P. citri* females. One of the possible reasons for its effectiveness would be its dual mode of action because Profenofos is a organophosphate inhibiting acetylcholinesterase (AChE), while the other insecticide, Cypermethrin, is a pyrethroid targeting sodium channel transmission of insect nervous system. Both of these insecticides are found effective against sucking insect pests such as whiteflies, aphids, jassids on cotton and hoppers on rice and mango plants (Rudramuni, 2011; Mansoor-ul-Hasan, 2000; Sandeep *et al*., 2006) [18, 20, 22]. Therefore, in spite of low absorption of target insecticides into female mealybug bodies, the joint action of two different insecticides might be lethal enough for these insect pests. These findings are in conformity with the results of Saeed *et al*. (2007) [21], Hussain *et al*. (2012) [13] and Gulzar *et al*. (2015) [12] who also demonstrated that profenofos is the most effective insecticide against mealybug infestation. Second most effective insecticide has been found Buprofezin which is an insect growth regulator and actually inhibits chitin biosynthesis. This IGR compound has already been claimed very effective against mealybugs and other homopteran insect pests along with Spirotetramat (Basit *et al*. 2012) [7]. These results are also in line with findings of Nikam *et al*., (2010) [19] under laboratory conditions.

However, the toxicity of imidacloprid and cypermethrin against *P. citri* has been found relatively lower than Polytrin-C and buprofezin in this study. One of the possible reasons for this ineffectiveness is potential development of insecticide resistance due to blind and irrational use these insecticides against mealybugs.

Conclusively, it is suggested that profenofos plus cypermethrin and buprofezin are good options to be incorporated as insecticidal control tools in the integrated management of mealybug menace to citrus production in the country. However, the bio-efficiency of these insecticides should be evaluated under field conditions because of the potential escape of mealybugs from applied insecticides either due to lower body penetration or obscuring nature of these insect pests (Kahramanoglu and Usanmaz, 2013) [14]. Moreover, the residual toxicity and non-target effects of these synthetic insecticides should also be determined under field conditions.

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