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In vitro analysis of toxic potential of systemic and contact insecticides on *Phenacoccus solenopsis* and its parasitoid *Aenasius* species

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Abstract

Cotton in Pakistan is sprayed heavily with different insecticide to have a control over pests. This experiment was conducted to check out the toxic potential of different systemic and contact insecticides on *Phenacoccus solenopsis* and its parasitoid *Aenasius* species. Nine doses in each twelve insecticides were evaluated under laboratory conditions against the living adults and immature of the *Aenasius* species. Insecticides were sprayed on adult's population by distributing them in an appliance enclosed with pesticide residues while the immature were kept in petri plates and sprayed by insecticides. Overall three insecticides; buprofezin (25% WP), esfenvelerate (5% EW) and leufenuron(EC) were found safety to parasitoid adults; whereas, , chlorpyifos (40 EC), cypermethrin (5 EC), emamectin Benzoate (l.9EC), esfensælerate (EW), Imidacloprid (20% SL), leufenuron (5% EC), nitenpyam (10 SL), profenofos (500 EC), pyriproxyfen (10.8 EC) and thiacloprid (480 SC) were ranked as safety for stages of parasitoid inside the mummy cases of the host. Acetamiprid (50% WDG) caused several mortality to both adult and immature stages of the parasitoid. On the basis of these finding, it was concluded that buprofezin (25% WP), esfenvelerate (5% EW) and leufenuron(EC) were safe in the field according to recommended doses for both of the adult and stages of parasitoid. Thus the mealybug parasitoids (*Aenasiu* ssp.) can be nominated in IPM programs of cotton mealybug.

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Introduction

Cotton (*Gossypium hirsutum* L.) is an important commercial non-food crop with global importance which contributes significantly to national economy (Nagrare *et al.,* 2009).Sucking pest of cotton like cotton mealybug (*Phenacoccus solenopsis)* damaged severely to cotton production.

The pests suck the cell sap, transmit diseases and lower the quality and productivity of cotton (Naqvi, 1976). Due to its devastating nature to damage, its role in crop failure is quite severe. Recently it had emerged as a serious threat to cotton production in Pakistan (Arif *et al.,* 2007). Mealybug excretes abundant honeydew on the surface of plant tissues which in turn leads to the development of many sooty molds that obviously hold back photosynthesis rate (Saeed *et al.,* 2007).Total cotton production reduced to 11.66 million bales against the target of 14.10 million bales because of mealybug's damage in Pakistan during 2006-2007 (Kakakhel, 2007).

Cotton in Pakistan is sprayed heavily with different insecticide to have a control over pests (Malik *et al.,* 1999).In the agro-ecosystem of cotton, about 70-80% of the imported insecticides are being sprayed to control sucking and chewing insect complex. These insecticides belong to OCs, OPs, carbamate, pyrethroids and others with various modes of action. The toxic residues of these insecticides are presented on the leaves/stems of treated plants cause toxicity in entomophagous insects wandering on plants in search of prey/hosts.

Paine *et. al.,* (2011) studied the potential effect of imidacloprid to parasitoid of cerambycid which damaged to *Eucalptus*. They treated the *Eucalptus rudis* tree at different formulations of imidacloprid and sampled nectars in five months after applications *Avetianellalongoi* that were fed floral nectar collected from treated trees had significantly lower survival and reproductive fitness than untreated trees. Prabhaker and Toscano (2007) evaluated the lethal effect of two insect growth regulators, *viz.* pyriproxyfen and buprofezin, and the effect of buprofezin was significantly greater when topical residual treatment was functional to the 1st instar compared to later instars. Pyriproxyfen had a dead effect on 1-2 days old but GWSS (glassy-winged sharpshooter) eggs did not impede late aged.

The present study was undertaken to assess the toxic potential of systematic and contact insecticides on *P. solenopsis* and its parasitoid *Aenasius* species. Nine doses including field recommended dose of each of the twelve insecticides (acetamiprid, emamectin benzoate, buprofezin, chlorpyrifos, cypermethrin, esfenvelerate, imidacloprid, leufenuron, profenofos, pyiproxyfen, nitenpyram and thiacloprid) were evaluated against free living adults and immature (present in mummy cases) of *Aenasiu* ssp. under laboratory conditions. The data regarding adult mortality of the parasitoid were recorded at an interval of 6hr for 24hr after treatment application but for immature (mummies) at an interval of 24hr for 25 days.

Materials and methods

Experiment was conducted in the eco-toxicology laboratory of the Department of Entomology, university of agriculture Faisalabad during the year 2014-2015.The major divisions of the whole experiment include.

Mass-rearing of cotton mealybug

Cotton mealybugs (along with infested stem) were collected from the infested shoe-flower plants (*Roza chinensis*) and placed in the transparent plastic-glass cages (1.3×0.9×1.3ft). These cages were provided with fresh pumpkin fruits as food for adult of female individuals as well as water and honey solution-socked cotton plug in petri dish as food for adult of male individuals of mealybug.

The cages were maintained under laboratory conditions for mass-rearing of mealybug at (28 ±1C, 70 ± 5% (RH) and 18 h with light /6 h with darkness).

Mass-rearing of cotton mealybug parasitoids, Aenasius species

Mummified individuals of mealybug were collected with camel brush and adult individuals of parasitoid were collected with aspirator from the infested shoe-flower plants. These individuals were collected on weekly basis and added to the cages to sustain their population.

Preparation of pesticide dilutions and replications

Treatments were include twelve insecticides such as, emamectin benzoate (1.9EC), leufenuron (5% EC), (25%), nitenpyram (10SL WP), pyriproxyfen (10.8EC), esfenvelerate (5% EW), acetamiprid (50% WDG), imidacloprid (20%SL), thiacloprid (480SC), chlorpyrifos (40 EC), buprofezin (25% WP), cypermethrin (5EC) and profenofos (500EC),were evaluated under laboratory conditions against free living adults and immature (mummy cases) of the *Aenasius* species using as the recommended dose, double of the recommended dose, 4 times, 8 times and 16 times of the recommended dose. Likewise half of the recommended doses, 1/4, 1/8, 1/16 of the recommended dose were used.

Insecticides test solutions/dilution

Different dilutions of the recommended doses of each insecticides such as 1/16×, 1/8×, 1/4×, 1/2×, 2×, 4×, 8× and 16× were prepared in water with the help of micropipettes. Nine test solutions of each insecticide were prepared and these test solutions were prepared from stock solution. Highest dose of stock solution (D-1) was prepared for each pesticide and serial dilutions were prepared by taking half of the stock solution and diluting it with distilled water to the original volume in another measuring cylinder to make D-2.

Bioassay procedure

The mummified mealybugs were transferred to petri dish containing moisture filter paper and untreated leaves. The insecticide was sprayed with atomizer on the leaves surface before mealybugs transferring and kept them under sunlight for 30 days. Two glass slides treated with pesticide were chosen for parasitoids exposure and fitted along with the transparent disposable cup containing tiny holes and a main hole at base for introduction of parasitoids. Hole was plugged with cotton and for three replicates; 10 units were prepared with dose rated and one control. Glass plates containing pesticide solution were dried for 25 min and affixed to inner wall of cups. Female adult of parasitoids were chosen for entry and kept them in an incubator under standard conditions, roughly checked with 6, 12, 18 and 24 hr interval. Mortality was corrected by Henderson and Tilton formula. 20 mummies were selected to check the role of insecticidal susceptibility and mummy case resistance. The petri dishes were sprayed with insecticide following a fine clay layer to prevent the exit of parasitoids. Incubator with controlled conditions was used for experiment replicated three times.

Statistical analysis

Data regarding the mortality of adults and immatures of the parasitoid of each experiment was analyzed statistically using Probit analysis technique (Finney, 1971) to determine LD50, chi-square (x^2), and confidence interval values. ANOVA technique and Tuckey Honestly difference test were also performed by Danho *et. al.*, (2002) to determine the significance of treatment applied and compare the means of parasitoid mortality for different treatments.

Results

Mortality percentage of parasitoid adults at different hours of exposure under FRD (Field recommended dose) different insecticides. The analysis reveals that mortality percentage of the adult stage of *Aenasius* species varied significantly during each interval of exposure time among different insecticides.The mortality response of adult parasitoids to different insecticides was increased along with the exposure time (Fig. 1).

After 6 hr of exposure

The results showed that the effect of different insecticide on the mortality percentage of the parasitoid adults was significantly different from each other at 6 hr (Fig. 1).

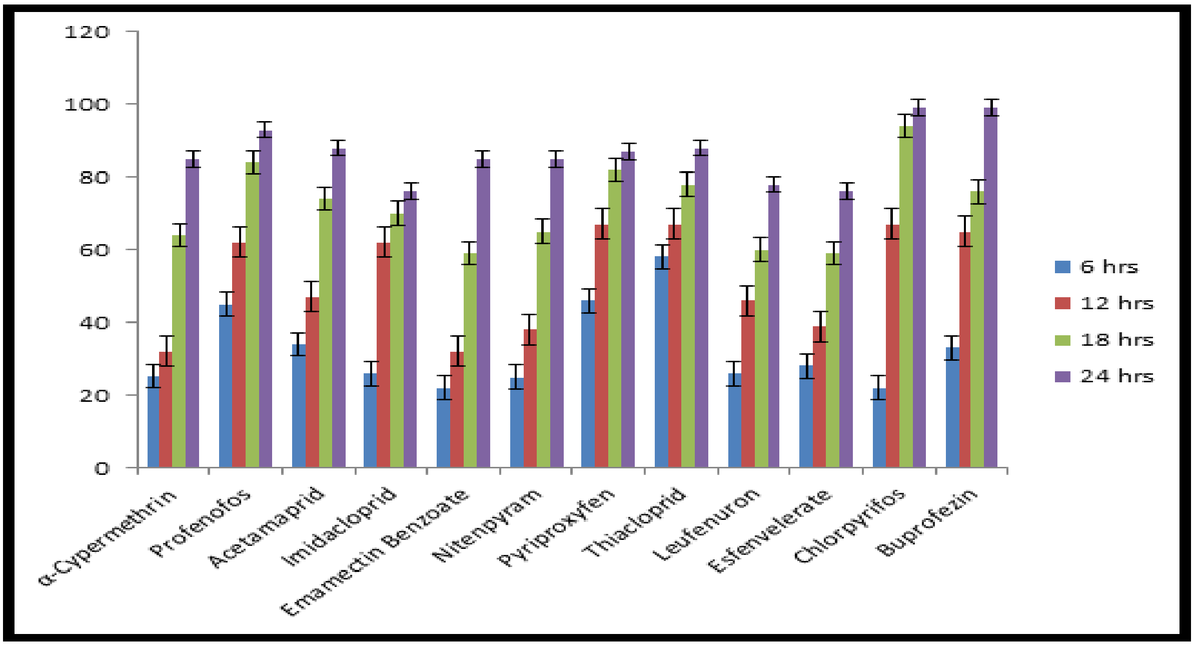

Fig. 1. Percentage mortality of the adult parasitoids by different insecticides after different exposure times.

Most of the tested insecticides caused less than 50% mortality (from 7% to 46%), except chlorpyrifos, which caused 65%. Thiacloprid(46%), nitenpyram (35%), cypermethrin (33%), acetamiprid (30%) and emamectinbenzoate (25%). Buprofezin and imidacloprid caused least only 7% and 12%mortality, and were safe for an exposure period of 6hr.

After 12 hr of exposure

Mortality percentage of the adults after 12 hrwas also significantly different from each other (Fig. 1). Most of the tested insecticides caused high mortality to the parasitoid population after exposure duration of 12 hr, except buprofezin, imidacloprid, leufenuron, and pyriproxyfen lower than 50%(21, 42, 32 and 49%). Chlorpyrifos caused maximum mortality of 90.7% to the parasitoid adult followed by acetamiprid (70%), thiacloprid (67%), emamectin benzoate (57%), nitenpyram (54%), cypermethrin (53%), profenofos (51%) and esfenvelerate (51%). These results depicted that buprofezin, leufenuron and imidaclopridwhich caused less mortality to adult parasitoids as compared to others.

After 18 hr of exposure

All the evaluated insecticides caused adult-parasitoid mortality in the range of 40.7% to 100%. Chlorpyrifos caused 100 % mortality to the adult parasitoid followed by acetamiprid (81%), pyriproxyfen (76%), cypennethrin (72%), thiaclopid (70%), emamectin benzoate (70%), imidacloprid (69%), profenofos (67%), nitenpyram (64%), esfenvelerate (62%), leufenuron (50%) and buprofezin (40.7%). Only buprofezin was the insecticide which caused mortality below 50 % (40.7 %) to the *Aenasius* species population. The results regarding the mortality of parasitoid-adults after 18 hr exposure revealed that the toxicity of insecticides residues persisted for 18 hr and parasitoid adult contacted the treated surface maximally after this exposure period. Over all, buprofezin and leufenuron were found less toxic to parasitoid adults comparatively after an exposure period of 18 hr.

After 24 hr of exposure

Most of the tested insecticides caused higher mortality to the parasitoid adult after 24 hr duration of exposure. All the tested insecticides, after this time interval, caused severe mortality in the range of 69-100%. Chlorpyrifos caused 100% mortality to the parasitoid, followed by acetamiprid (98%), cypermethrin (88%), pyriproxyfen (86%), emamectin benzoate (81%), profenofos (8%), thiacloprid, (77%), esfenvelerate (74%), imidacloprid (74%), leufenuron (74%), nitenpyram (70%) and buprofezin (69%). The results regarding the mortality of parasitoid-adults after 24 hr exposure revealed that the toxicity of insecticides residues persisted for 24 hr and the contact of the parasitoid adult with treated surface enhanced after this exposure period.

Discussion

Three insecticides, buprofezin (25% WP), esfenvelerate (5% EW) and leufenuron (5% EC) were found safe for parasitoid adults. Buprofezin (25% WP) killed most of the sucking pests of cotton by inhibiting chitin biosynthesis (Izawa *et al.,* 1997). Cloyd and Dickinson(2006)exposed that adults and the immature stages of the parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), natural enemies of citrus mealybug, *Planococcus citri* (Homoptera: Pseudococcidae) to the pesticide residues and found that the insecticide was least toxic to the parasitoid. Cordero *et al.* (2007) conducted a laboratory experiment to bioassay the impact of series concentrations of esfenvelerate on the adults of *Diadegma insulare* and *Oomyzus sokolowskii* (two important parasitoids of diamond back moth *Plutella xylostella*). The results showed that the insecticide was toxic to both parasitoids and that field application would impact biological control of diamond back moth. The results of present study showed that leufenuron caused non-significant mortality in 6-12 hr of exposure. After the exposure for 18 hrs the FRD caused 50% mortality of all the other doses non-significant. After 24 hrs, FRD caused 72 % and all the doses caused complete mortality but still the effect of all the lower doses was non The probit analysis of the current study regarding the effect of Leufenuroa on the mortality of the adult of the mealybug parasitoid, *Aenasius* species Also indicates that the insecticide is non-lethal to the parasitoid for the early duration of 18 hrs but the insecticide became lethal the passage of the next 6hrs.Pyriproxyfen (10.8EC), thiacloprid (450SC), profenofos (500 EC), buprofezin (25% WP), imidacloprid (20% SL), cypermethrin (5% EC), chlorpyrifos (40% EC), emamectin benzoate (1.9EC), esfenvelerate (5% EW), leufenuron (5% EC) and nitenpyram (10SL) were ranked as safety for immature stages of parasitoid inside the mummy cases of the host.

Darriet *et al.* (2010) evaluated the operational efficiency of a mixture composed of pyriproxyfcn (an insect growth regulator) and spinosad (a biopesticide) against a population of *Aedes aegypti.* The results showed that the mixture of pyriproxyfen + spinosad remained active for at least 8 months, compared with 3 months for spinosad alone, and 5 months for pyriproxyfen alone. Thiacloprid caused significant mortality in the 6hrs exposure interval. FRD caused 89 % mortality after 12hrs and all the higher doses caused complete mortality of 100 %. After 18 hrs exposure, FRD/2 also caused 88.9 % mortality and all the above doses caused 100 % mortality after 24 hrs, rest of the doses caused non-significant mortality. Profenofos is extremely toxic, broad spectrum insecticide. Significant mortality was recorded under 4FRD and higher doses of the profenofos after 6hrs of exposure. FRD caused 22 % mortality while all the higher doses caused 50% mortality. Similar results were obtained by Khan *et al.*(2005) who found profenofos to be very toxic to the adults of the parasitoid *Braconhabitor*. The results of the probit analysis regarding the data on the effects of Profenofos on the adults of the *Aenasius* also revealed that profenofos was moderately toxic in the half passed time of the trial. Imidacloprid mortality was significant after 6 hrs of exposure, when highest mortality caused by 8FRD (44.43 %) in the next 6 hrs all the doses caused dose caused only 11%. Similar results were obtained after 24 hrsexposure. Paine *et al.,* (2011) found the same result during evaluating the potential risks of systemic to enemies of *cerambycid* attacking *Eucalyptus.*

Mortality caused by cypernethrin was significantly high With 12 hrs exposure to residues, mortality caused by FRD reached up to 89 % and all the other doses to FRD. All the doses caused complete mortality after 24 hrs however lower dose (0.1S62SmVL) caused 39 % mortality. Mgocheki and Addison (2009) conducted a bioassay in which they found the insecticide to cause acute mortality to of the parasitoids, *Anagyrus* species.

Chlorpyrifos (40 EC) causes mortality by contact and stomach action with longer residually. Most of the doses tested in this bioassay caused 50 % mortality just after the first six hours. The mortality reached to 100% or near this in the next 6hrs under all most all of the doses. Khan *et al.*, (2005) during an experiment exposed the same result.

Emamectin Benzoate (1.9 EC) is non-systemic insecticide which penetrates leaf tissues by translaminar movement the results showed, the pesticide (causing < 50% mortality) only after the duration of first 6 hrs under the FRD and the lower doses tested but when the adults were further exposed to the insecticide residues for 12FRDand all the higher doses become lethal (mortality > 50%) to parasitoid. After 18 and 24 hrs all of the doses became lethal to the parasitoid. Analysis also suggest the pesticide to be safe for the parasitoid only if the parasitoid is exposed to the pesticide residues for just 6 hrs (LD_{50} > FRD). The insecticide dangerous/ lethal to the pesticide, when the adults of the parasitoid were further exposed for duration of 12, 18 and 24 hrs (LD_{50}< FRD) as the facts showed. Khan *et al.* (2005) (double) found the pesticide to be slightly toxic to the parasitoid *(Bracon habitor)*.

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