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Original Article

Determination of hormoligosis of organophosphate insecticides against *Phenacoccus solenopsis*

Determinação da hormoligose de inseticidas organofosforados contra *Phenacoccus solenopsis*

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Abstract

Cotton mealybug is a highly invasive pest of agricultural crops worldwide. Major agriculturists most rely on the use of insecticides for the control of pesticides. So, the indiscriminate use of insecticides leads to resistance development in recent years. For this purpose, an experiment was conducted using different concentrations of the three insecticides (profenfos chlorpyrifos and triazophos) to check the hormoligosis effects against cotton mealybug (CMB) in laboratory conditions. Investigation of variations for % mortality of adults of CMB after three days revealed that all treatments had statistically significant ($P < 0.05$). The highest mortality was observed at the highest concentrations of profenofos 2.4% (38.55%). After 7 days, all the treatments were significant with difference in means ($P < 0.05$). The highest mortality was recorded at the highest dilution of pesticide profenofos 2.4% (77.11%). The values of fecundity and longevity exposed a valid difference among treatments ($P < 0.05$). Maximum fecundity was observed at the concentration 2.4% (181.41%) and longevity showed (38.46%). The highest mortality was observed at a concentration of triazophos 4% (27.98%). For chlorpyriphos the highest mortality was examined at concentration 4% (24.79%). The fecundity showed a statistically significant difference for different concentrations of triazophos and chlorpyriphos ($P < 0.05$). The results of the recent study provide valuable information regarding the selection of insecticides and hormoligosis effects. The study can be helpful in the implications of integrated pest management of *P. solenopsis.*

Keywords: organophosphate, resistance development, pest management, cotton pest.

Resumo

A cochonilha-do-algodão é uma praga altamente invasiva das culturas agrícolas em todo o mundo. Os grandes agricultores dependem muito mais do uso de inseticidas para o controle de pesticidas. Assim, o uso indiscriminado de inseticidas tem levado ao desenvolvimento de resistência nos últimos anos. Para tanto, foi realizado um experimento utilizando diferentes concentrações de três inseticidas (profenofós, clorpirifós e triazofós) para verificar os efeitos da hormoligose contra a cochonilha-do-algodão (CMB) em condições de laboratório. A investigação das variações para % de mortalidade de adultos de CMB após três dias revelou que todos os tratamentos tiveram significância estatística ($P < 0,05$). A maior mortalidade foi observada nas maiores concentrações de profenofós a 2,4% (38,55%). Após 7 dias, todos os tratamentos foram significativos com diferença nas médias ($P < 0,05$). A maior mortalidade foi registrada na maior diluição do agrotóxico profenofós a 2,4% (77,11%). Os valores de fecundidade e longevidade expuseram uma diferença válida entre os tratamentos ($P < 0,05$). A fecundidade máxima (181,41%) e a longevidade (38,46%) foram observadas na concentração de 2,4%. A maior mortalidade foi observada na concentração de triazofós a 4% (27,98%). Para o clorpirifós, a maior mortalidade foi percebida na concentração de 4% (24,79%). A fecundidade apresentou diferença estatisticamente significante para as diferentes concentrações de triazofós e clorpirifós ($P < 0,05$). Os resultados do recente estudo fornecem informações valiosas sobre a seleção de inseticidas e os efeitos da hormoligose. O estudo pode ser útil nas implicações do manejo integrado de pragas de *Phenacoccus solenopsis*.

Palavras-chave: organofosforados, desenvolvimento de resistência, manejo de pragas, praga do algodão.

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1. Introduction

In Pakistan, agribusiness is one of the most prominent business which contributes twenty-one percent to GDP and utilizes forty-four percent of the workforce. *Gossypium hirsutum* is among the top agribusiness, and its fiber is considered adequate in the textile industry. Pakistan is among the top ten cotton producers globally (Ashraf et al., 2018). It is cultivated in two big provinces, namely Sindh and Punjab. However, cotton harvest is harmed by many sucking and bollworms insects pests species, and inappropriate management of these insects results in a significant economic loss (Khan et al., 2020; Waqas et al., 2021).

Phenacoccus solenopsis commonly known as cotton mealybug (CMB), a potential insect of *G. hirsutum*. It attacks plants and sucks cell sap from shoots, fruiting plants, branches, and leaves. CMB discharge excess sugars like honeydew can disable photosynthetic efficiency (Nagrare et al., 2020) and has caused a decline in cotton yield in China, India, and Egypt (Yang et al., 2012; Jabran and Chauhan, 2019; Rezk et al., 2019; Chen et al., 2021). In addition, there is a large effect of CMB on *G. hirsutum* harvesting. In India and Thailand, CMB infested 30-40% cotton plants leading to a reduction in overall cotton yield (Tanwar et al., 2011). In Punjab, the infestation in 2006 caused by the CMB on the cotton crop was almost twelve percent, and the ratio of the infestation increased in 2007 reached about 40% (Nagrare et al., 2009; Waqas et al., 2021). Thus, the species has a substantial economic threat to cotton, and other agriculture crops worldwide, particularly in tropical regions (Nagrare et al., 2016).

Many pesticides have been used to control the infestation of cotton mealybug, but their high concentrations caused the resistance. Now in the era of integrated pests management, the use of pesticides should be in a proper dose selection to avoid the effect on other non-targeted species (Biondi et al., 2012). To overcome the effect of CMB on plants, different types of pesticides are used. These pesticides cause retardation in physiology, morphology, reproduction, and growth. When the insects are subjected to low and high pesticide solutions, it causes damage and helps insects develop resistance against them (Nagrare et al., 2020). Therefore, to minimize the resistance, a new phenomenon is introduced, known as hormoligosis. A mixture of different pesticides is now being used against insect pests. Some researchers used hormoligosis of pesticides including Spinosad, buprofezin, chlorpyrifos-methyl and pyriproxyfen against the natural enemies of citrus scale pests (Siscaro et al., 2006; Suma et al., 2009). For a sustainable ecosystem, it is dire to control the harmful effects of pesticides. They can select their desired insect and produces minimum harm to the Agro-ecosystem (Barbosa et al., 2018). This study was executed from the above-discussed information to assess the hormoligosis of three organophosphate pesticides against CMB under laboratory situations.

2. Materials and Methods

2.1. Mass-rearing of P. solenopsis

P. solenopsis was sampled from *G. hirsutum* infected stem and leaves and put in a visible jar. The female was provided with clean pumpkin (containing no pesticide effect before its use) while males were provided cotton plug saturated with the honey-water solution as a diet. Jars were then kept in laboratory conditions (50% relative humidity and 25 ± 1 °C temperature) for the mass-culturing of the mealybug. After the rearing of new culture wild population was removed from the mass.

2.2. Preparation of pesticides test solutions

Three organophosphate pesticides chlorpyrifos, profenofos, and triazophos were purchased from the grain market Faisalabad and used to prepare for different solutions. Five dilutions of every pesticide were arranged from standard dilution (Table 1). The standard dilution of the maximum concentration was formed from every pesticide. Successive solutions were prepared by taking 1/2 of standard dilutions and reducing it by the purified H_2O to a new volume in a different measuring cylinder to make another solution. Consecutive solutions were prepared with the help of this technique until the 5th solution for every pesticide.

Table 1. Dilutions of pesticide tested in present experiment.

Name of pesticides	Active ingredient	Formulation	Company	Concentration
Polytrin C	Profenofos Cypermethrin	440 EC	Syngenta Warehouse Plot no 90, Industrial Area, Kot Lakhpat Lahore	0.15%,0.3%,0.6%, 1.2%, 2.4%
Chlorpyrifos	Chlorpyrifos	40 EC	Vantage Chemicals [Pvt.] Ltd.39BIII, M.A, Johar Town Lahore.	0.25%,0.5%, 1%, 2%, 4%
Ecophos	Triazophos	40 EC	M/s. Tara Imperial Industries (Pvt.) Ltd., Chunnai Bypass Road, Raiwind, 28KM Thokar Niaz Baig, Lahore.	0.25%,0.5%,1%, 2%, 4%

(EC = Emulsifiable Concentrate).

2.3. Layout of experiment

The pumpkin was washed with H_2O and stored in the laboratory for the complete evaporation of H_2O from its surface. After that, five dilutions were prepared to form the standard dilutions. Then the pumpkin was sprayed with all test dilution of each pesticide in the laboratory and dried it on the filter paper. The experimental component having 1 pumpkin sprayed with specific pesticide dilutions was produced. Twenty individuals of adult CMB were collected from susceptible culture and transferred to the pesticides prayed pumpkin by camel hairbrush. To assess the fecundity 20 couples were placed in the plastic pot and feed with diet. The CMB were offered to permit the egg production. After the production eggs were counted. For the longevity measurement, 20 individuals were again selected and placed in the plastic jars. After the insecticides exposure the individuals were counted until their death. Control group of CMB for the mortality, fecundity and longevity was also managed with no pesticide exposure (only pumpkin was provided as diet). The exposed individuals were moved to petri-dish and deliberately observed below a microscope. The individuals showed no locomotion was observed dead. The data of CMB mortality was accumulated after three and seven days.

2.4. Statistical analysis

The data was composed of dead individuals which then changed into % corrected mortality using the Abbott formula (Abbott, 1925). Ultimately, the whole data was exposed to ANOVA and Tuckey HSD test to difference of means. The data was analyzed at the level of 0.05.

3. Results

3.1. Percent mortality of adult CMB at various dilution of profenofos following three days and seven days of applications

The mortality of cotton mealy bug (CMB) after three days, revealed that all treatments had statistically considerable differences (P < 0.05). Highest mortality was observed at highest solutions of profenofos 2.4% (38.55%) followed by 1.2% (27.88%), 0.6% (20.42%), 0.3% (13.55%), 0.15% (7.52%) respectively (Table 2). After 7 days all the treatments were statistically significant with different values (P < 0.05). Highest mortality was recorded at highest dilution of pesticide profenofos 2.4% (77.11%) followed by 1.2% (68.32%), 0.6% (62.03%), 0.3% (53.49%), 0.15% (36.08%) respectively (Table 2).

3.2. Fecundity and Longevity of CMB at different dilutions of profenofos treatments

The values of fecundity showed the significant difference among treatments (P < 0.05). Maximum fecundity was observed at the highest concentration of profenofos 2.4% (181.41%) followed by 1.2% (163.34%), 0.6% (153.33%), 0.3% (146.31%), 0.15% (141.18%) respectively (Table 3). Analysis of variance for longevity showed that all the treatments had statistically significant difference (P < 0.05). Maximum longevity was observed at the highest concentration of profenofos 2.4% (38.46%) followed by 1.2% (34.21%), 0.6% (30.52%), 0.3% (26.86%), 0.15% (24.97%) respectively (Table 3).

3.3. Percent mortality of CMB at different dilutions of triazophos and chlorpyriphos after three and seven-days treatments

Analysis of variance for percent mortality of CMB after 3 days shows that all the applications had statistically notable difference (P < 0.05). Highest mortality was examined at the highest dilutions of triazophos 4% (27.98%) followed by 2% (20.89%), 1% (17.02%), 0.5% (13.78%), 0.25% (3.62%) respectively. Percent mortality after 7 days showed statistically significant difference among all the treatments (P < 0.05). Highest mortality was examined at the highest dilutions of triazophos 4% (68.67%) followed by 2% (63.99%), 1% (57.12%), 0.5% (49.33%), 0.25% (44.98%) respectively (Table 4). After 3 days results revealed that all the applications had statistically notable (P < 0.05). Highest mortality was examined at the highest dilution of chlorpyriphos 4% (24.79%) followed by 2% (22.22%), 1% (14.82%), 0.5% (10.72%), 0.25% (8.22%) respectively. CMB after 7 days revealed that all the applications had statistically significant deviation (P < 0.05). Highest

Table 2. Means comparison for percent mortality of adults of CMB at different dilutions of profenofos after three days and seven days of treatments.

Concentration (%)	Mean ± SE	Mean ± SE
	After three days of treatment	After seven days of treatment
0.15	7.52 ± 0.84[e]	36.08 ± 8.25[b]
0.3	13.55 ± 0.92[d]	53.49 ± 7.55[ab]
0.6	20.42 ± 0.35[c]	62.03 ± 9.35[ab]
1.2	27.88 ± 0.65[b]	68.32 ± 6.95[a]
2.4	38.35 ± 0.87[a]	77.11 ± 7.85[a]
Control	6.55 ± 0.45[e]	4.42 ± 9.75[c]

Means that don't share similar letters are significant. (SE = Standard Error).

Table 3. Means comparison for the fecundity and longevity of CMB at different dilutions of profenofos treatments.

Concentration (%)	Fecundity	Longevity
	Mean ± SE	Mean ± SE
0.15	141.18 ± 4.27[c]	24.97 ± 2.78[c]
0.3	146.31 ± 4.47[c]	26.86 ± 2.58[c]
0.6	153.33 ± 4.67[bc]	30.52 ± 2.88[bc]
1.2	163.34 ± 4.37[b]	34.21 ± 2.48[bc]
2.4	181.41 ± 4.77[a]	38.46 ± 2.98[ab]
Control	92.42 ± 4.17[a]	46.68 ± 2.38[a]

Means that don't share similar letters are significant.

Table 4. Means comparison for percent mortality of CMB at different concentrations of triazophos and Chlorpyriphos after three and seven days of treatments.

Concentration (%)	Triazophos		Chlorpyriphos	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
	After three days of treatments	After seven days of treatments	After three days of treatment	After seven days of treatment
0.25	3.62 ± 0.78ᵉ	44.98 ± 0.59ᵉ	8.22 ± 0.53ᵉ	12.38 ± 0.68ᵉ
0.5	13.78 ± 0.88ᵈ	49.33 ± 0.79ᵈ	10.72 ± 0.73ᵈ	19.09 ± 0.65ᵈ
1.00	17.02 ± 0.58ᶜ	57.12 ± 0.49ᶜ	14.82 ± 0.65ᶜ	26.00 ± 0.45ᶜ
2.00	20.89 ± 0.48ᵇ	63.99 ± 0.69ᵇ	22.22 ± 0.33ᵇ	28.49 ± 0.75ᵇ
4.00	27.98 ± 0.68ᵃ	68.67 ± 0.39ᵃ	24.79 ± 0.43ᵃ	38.00 ± 0.55ᵃ
Control	6.22 ± 0.38ᵉ	7.92 ± 0.89ᶠ	4.65 ± 0.57ᶠ	6.65 ± 0.35ᶠ

Means that don't share similar letters are significant.

mortality was observed at the highest concentration of chlorpyriphos 4% (38.00%) followed by 2% (28.49%), 1% (26.00%), 0.5% (19.09%), 0.25% (12.38%) respectively (Table 4).

3.4. Fecundity of CMB at different dilutions of triazophos and chlorpyriphos of treatments

The results of fecundity showed that treatments had statistically significant difference (P < 0.05). Maximum fecundity was observed at the highest concentration of triazophos 4% (176.08%) followed by 2% (161.65%), 1% (145.92%), 0.5% (141.09%), 0.25% (135.95%) respectively (Table 5). Analysis of variance for fecundity showed that all the treatments had statistically significance (P < 0.05). Maximum fecundity was observed at the highest concentration of chlorpyriphos 4% (156.35%) followed by 2% (148.30%), 1% (139.56%), 0.5% (134.13%), 0.25% (130.14%) respectively (Table 5).

3.5. Longevity of CMB at different dilutions of triazophos and chlorpyriphos treatment

The longevity showed statistically significant difference among all means (P < 0.05). Maximum longevity was observed at the highest concentration of triazophos 4% (36.91%) followed by 2% (32.82%), 1% (27.78%), 0.5% (25.55%), 0.25% (22.71%) respectively (Table 6). Analysis of variance for longevity showed that all the treatments had statistically significant with difference (P < 0.05). Maximum mortality of CMB was observed at the highest concentration of chlorpyriphos 4% (31.59%) followed by 2% (27.81%), 1% (24.19%), 0.5% (21.95%), 0.25% (19.40%) respectively (Table 6).

Table 5. Comparison means for the fecundity of CMB at different dilutions of triazophos and Chlorpyriphos treatments.

Concentration (%)	Triazophos	Chlorpyriphos
	Mean ± SE	Mean ± SE
0.25	135.95 ± 6.55ᵈ	130.14± 6.21ᶜ
0.50	141.09 ± 6.78ᶜᵈ	134.13 ± 6.36ᶜ
1.00	145.92 ± 6.48ᶜᵈ	139.56 ± 6.24ᵇᶜ
2.00	161.65 ± 6.88ᵇᶜ	148.30 ± 6.33ᵃᵇᶜ
4.00	176.08 ± 6.28ᵃᵇ	156.35± 6.45ᵃᵇ
Control	188.42 ± 6.98ᵃ	166.48 6.38ᵃ

Means that don't share similar letters are significant.

Table 6. Means for the longevity of CMB at different dilutions of triazophos and Chlorpyriphos treatments.

Concentration (%)	Triazophos	Chlorpyriphos
	Mean ± SE	Mean ± SE
0.25	22.71 ± 2.37ᵈ	19.40 ± 2.20ᵈ
0.50	25.55 ± 2.347ᶜᵈ	21.95 ± 2.30ᶜᵈ
1.00	27.78 ± 2.77ᶜᵈ	24.19 ± 2.37ᵇᶜᵈ
2.00	32.82 ± 2.27ᵇᶜ	27.81 ± 2.40ᵇᶜ
4.00	36.91 ± 2.57ᵃᵇ	31.59 ± 2.45ᵃᵇ
Control	43.55 ± 2.67ᵃ	35.66 2.50ᵃ

Means that don't share similar letters are significant.

4. Discussion

In Pakistan, since 2005, cotton mealybug has been considered a sucking insect pest on cultured cotton crops (Abbas et al., 2010; Joshi et al., 2010). Insect Growth Regulators appears the newest for the commercial and operational pest control. Their species are more than conventional pesticides suggested a good substitute for selective insect control in harmony with present Integrated Pest Management programs. Insect growth regulators normally have a good boundary of care for most non-target environment like birds, fishes, invertebrates and other wildlife. Unfortunately, few biorationals offer effective control of target pests like mealy bugs and selectivity to many beneficial insects (Jhala et al., 2010).

The results showed that concentration-dependent percent mortality of CMB decreased with decreased concentration after three days. While on the contrary, after seven days mortality was increased when concentration decreased at different dilutions of polytrin®, ecophos® and chlorpyriphos® pesticides, which shows that growth of resistance is not produced. Present findings are supported by several studies where profenofos, triazophos and carbaryl were used against the CMB in which maximum mortality was caused by the profenofos (Jhala et al., 2010; Nagrare et al., 2016). In literature, it was observed that sublethal doses had different impacts on the growth period of beetles compared with that of the control that sublethal concentrations of profenofos have negatively damaged the biological and development operations (Singh et al., 2017).

The lethal and sub-lethal effects of many insecticides such as triazophos, carbaryl and profenophos (via the acetylcholinesterase enzyme inhibition) are considered most successful for cotton pests in field and laboratory conditions (Jhala et al., 2010; Saeed et al., 2017, 2021). Vojoudi et al. (2011) experimented to examine the lethal with sub-lethal outcomes of some biorational on 3rd instars larvae at various stages of cotton bollworm and its mortality, fecundity and longevity. They concluded that spinosad and chlorpyrifos were more functional pesticides against the cotton bollworm than the abamectin. Fernandes et al. (2016) performed an experiment to evaluate the sub-lethal doses with lethal effects on non-target and target arthropods such as ladybird beetle, flower bug and soldier beetle. These 3 species were exposed against the pesticides such as imidacloprid, bifenthrin, chlorpyrifos, acephate and chlorantraniliprol to check the impacts of these pesticides. They concluded that acephate and chlorpyrifos were more functional as compared to the chlorantraniliprol.

In the present study, maximum fecundity of CMB was observed at concentration of triazophos and chlorpyriphos 4% (176.08%), (156.35%), respectively. Maximum longevity was observed at the highest concentration of triazophos and chlorpyriphos 4% (36.91%), (31.59%), respectively. The longevity also increased in the case of polytrin®, ecophos® and chlorpyriphos® throughout the generations. Present findings are in parallel work carried out by Carneiro et al. (2014) as they checked the efficacy of abamectin, chlorpyriphos, and Spinosad on the longevity of cotton bollworm. They found chlorpyriphos significantly influence cotton bollworm's longevity compared to Spinosad and abamectin (Carneiro et al., 2014). Another study tested three doses of Spinosad against 3rd and 4th instar of *S. litura* to check mortality, adult emergence, and pupation. It was observed that Spinosad has a synergist effect result in high mortality and low adult emergence (Ahmad et al., 2020). The resistance step to pesticides negatively impacts the level of development on reproductive potential as minimum improvement was observed in our study for strains with a maximum resistance period. However, profenofos could be more suitable than chlorpyriphos and triazophos to manage this pest based on sublethal and lethal impacts.

5. Conclusion

The present study concluded that the overall use of insecticides prevail resistance in pest control. The hormoligosis results showed that alternative and sublethal doses of insecticides can be useful in the decrease of resistance development. In the present study observation, profenophos insecticide showed better results in the control of resistance during application and it also has been proved that use of sublethal doses could be possible solution. The profenophos resulted > 90% mortality against *P. solenopsis*. These results can be used for the future studies and application of insecticides in the crops.

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