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Research Article

Laboratory Evaluation of Toxic Potential of Different Insecticides Against Pink Bollworm (*Pectinophora gossypiella*)

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MUA and RMM designed the study. MUA conducted the study. MUA and RMM analyzed the data and wrote the manuscript.

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Insecticide efficacy, Bioassay, Pyrethroids, Organophosphates

Abstract | Toxic potential of different insecticides *i.e.*, Chlorpyrifos 40 EC, Polytrin-C 440 EC, Match 5.0 EC, Karate 2.5 EC and Proclaim 1.9 EC was evaluated against 3rd instar larvae of pink bollworm (*Pectinophora gossypiella*) at their recommended and low doses by using boll dip approach under lab conditions. The outcomes revealed that after 24 h of treatment Polytrin-C 440 EC showed highly significant toxicity with 93.33% lethality to the pink bollworm followed by Proclaim 1.9 EC and Chlorpyrifos 40 EC causing 80% mortality at their recommended doses. After 48 h, 100% mortality was recorded in Polytrin-C 440 EC and Proclaim 1.9 EC on their recommended doses whereas percent mortality was reached to 86.66% in Chlorpyrifos 40 EC. However, least effectiveness was shown by Match 5.0 EC on its recommended dose with 46.66% lethality after 24 h and 53.33% lethality after 48 and 72 h of exposure. Furthermore, Polytrin-C 440 EC also showed highest mortality on its low dose with 53.33% and 73.33% mortality after 24 and 48 h of exposure, respectively. It is thus concluded that Polytrin-C 440 EC is the most effective insecticide against pink bollworm among the other evaluated insecticides.

Novelty Statement | This study established local base line susceptibility of the target insect pest population in Pakistan.

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Introduction

Cotton (*Gossypium hirsutum* L.) is a pivotal economic crop that provides necessary commodities to the textile and other various sectors. Pakistan ranks sixth cotton producer worldwide and its value accounts for 2.9% in Pakistan's agriculture sector, 0.7% of its GDP and 51 % of its external income (GOP, 2024). Several insect pest species targets cotton crop from germination phase up to the time of picking (Sarwar and Sattar, 2013). Lepidopteran pests belonging to family Noctuidae and Gelechiidae are especially detrimental ones (Tabashnik *et al*., 2002).

Amid numerous others, pink bollworm is considered foremost economically damaging insect pest of cotton, present specifically in Australia, USA, Africa and Asia (Wan *et al*., 2017; Naik *et al*., 2018). Females oviposit 100 - 250 eggs solely on the new sprouts, beneath the leaves, floral buds and developing bolls. The larval stage feeds on reproductive structures of cotton plant. Its foraging within the green bolls contributes to decay, malformation, and partial boll opening, as well as lint staining (yellowing),

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all of which reduce fiber quality and length (Ishtiaq *et al*., 2024). Based on the magnitude of infestation and weather scenario, this pest may lead to 20-30% yield loss. It is estimated that Pakistan incurs annual loss of one million bales due to the attack of pink bollworm. It has caused severe damage in 1965, 1983 and 2015-2019 (Arshad, 2020).

Synthetic insecticides application is the most effective technique for insect pest control employed all around the globe. Since the 1960s, worldwide pesticide use has increased manifold (Sheikh *et al*., 2011). The reliance on insecticides in Pakistan is strikingly obvious from its rapidly increasing pattern of consumption, i.e. the pesticide imports enhanced to 37,000 tons in 2023 from 13,030 tons in 1990 (Tauseef *et al*., 2024). Monetary significance of produce urged the farmers to apply insecticides very frequently, sometimes twice the recommended dose (Babar *et al*., 2016). At present a huge quantity of synthetic chemicals is being used to overcome devastating pests compared to earlier times (Rauf *et al*., 2019).

However, insecticide application often fails because larvae complete development inside bolls (Fand *et al*., 2020; CABI, 2022). They can even survive within rosette flowers, hence guarded from the immediate insecticide contact leading to recurring applications by the growers for achieving the pest control at optimal level. Such repeated applications eventually exacerbated problems like pollution, higher costs, resistance (Bajya *et al*., 2010).

Key components to manage insecticide resistance includes reducing insecticide usage, refrain from tank mixtures, evading persistent compounds and avoiding repeated usage of insecticides from same groups (Bethke, 2010). Through these tactics, tolerance could be retarded or declined through maintaining sufficient abundance of susceptible organisms via utilization of insecticides at minimum doses and evasion in the assortment of heterozygous carrier of resistance allele. Conversely, it is also advised to use pesticides at higher rates where resistance is dominant although restricted to lessen or diminish the presence of heterozygotes (Helps *et al*., 2017). Many scientists conducted experiments on this perspective with fungicide and rodenticides, whereas only few were carried out to manage resistance by high/low dose tactic against insects (Denholm and Rowland, 1992).

The monitoring techniques, strict surveillance, synchronized timing of insecticide spraying with insect pest abundance, etc., is highly significant. Calendar-based sprays are unwise once larvae enter the bolls due to the inaccessibility of the target site. Thus, for achieving high efficiency of control the insecticides should be applied before boll entry (Fand *et al*., 2021; Fand, 2021).

Given this scenario, we evaluated five insecticides at recommended and half rates against 3rd-instar PBW using a boll-dip bioassay.

Materials and Methods

Insect used

The pink bollworm larvae were collected from the cotton crop raised at the research area of Nuclear Institute of Agriculture (NIA), Tandojam during the Kharif season of 2024. Destructive sampling method was used for collection of pink bollworm in required quantity from naturally infested green bolls. Bolls manifesting natural infestation symptoms were detached and brought to the laboratory, where they were dissected to recover the larvae from inside. All the treatments of insecticides were evaluated on the 3rd larval instar obtained from the dissected bolls. Experiments were performed in maintained laboratory conditions at 25 $\pm$ 2 °C, 60 $\pm$ 5% RH and 14-10 h (L:D) photoperiod.

Insecticides

Different insecticides *viz*., Chlorpyrifos 40 EC, Polytrin-C 440 EC, Match 5.0 EC, Karate 2.5 EC and Proclaim 1.9 EC were evaluated for their toxic potential against natural field population of 3rd instar larvae pink bollworm to develop baseline susceptibility data. Two dosages of each chemical i.e., low dose (half of recommended amount) and recommended dose as mentioned on label were prepared for toxicity evaluation (Table 1).

Toxicity test

In this experiment, green bolls were pre-treated with insecticide dilutions before the release of larvae. Healthy and fresh green bolls displaying no symptoms of infestation were collected from the cotton crop and brought to the laboratory where they were washed with water for removal of any dust particles and dried. Bolls were dipped in each insecticidal solution for 10 s and then dried on the blotting paper. The bolls treated with insecticide were kept in small glass jars covered with muslin cloth. The larvae were starved for 6 h before being released into the glass jars. Five larvae were released into each jar containing three treated bolls. The treated jars were arranged randomly during experiment. Total fifteen larvae and nine green bolls were used in three replications for toxicity evaluation of each insecticidal treatment. In the control, bolls were dipped in the distilled water and the larvae were released for comparison.

Statistical analysis

The observations on lethality of larva were noted after 24, 48, and 72 h of exposure. Larvae were declared killed if they gave no response to stimulation by touch. Data on percent larval mortality was subjected to statistical analysis

Table 1: Details of insecticides.

Tr.	Insecticides	Active ingredient	Mode of action (IRAC classification)	Dose/acre (ml/100 L)		Active Ingredient in final dilution (g/ml)*	
				Low	Recommended	Low	Recommended
T1	Chlorpyrifos 40 EC	Chlorpyrifos	Acetyl cholinesterase (AChE) inhibitors	500	1000	0.2	0.4
T2	Polytrin-C 440 EC	Profenofos + Cypermethrin	Acetyl cholinesterase (AChE) inhibitors + Sodium channel modulators	300	600	0.22	0.44
T3	Proclaim 1.9 EC	Emamectin Benzoate	Glutamate-gated chloride channel (GluCl) allosteric modulators	100	200	0.0096	0.0192
T4	Karate 2.5 EC	Lambda cyhalothrin	Sodium channel modulators	165	330	0.0125	0.025
T5	Match 5.0 EC	Lufenuron	Inhibitors of chitin biosynthesis affecting CHS1	100	200	0.025	0.05
T6	Control						

*Insecticides treatments were prepared in one litter. Note: Insecticides used for low and recommended doses (ml); T_1: 5 and 10, T_2: 3 and 6, T_3: 1 and 2, T_4: 1.65 and 3.3, T_5: 1 and 2.

through one way ANOVA at each time point and LSD test ($p \leq 0.05$) was used for means comparison by using computer software Statistix 8.1.

Results

Percent mortality at recommended doses

The outcomes regarding percent larval mortality of pink bollworm after exposure to recommended doses at various time intervals manifested substantial differences (Table 2). The highest percentage mortality of 93.33% was noticed in Polytrin-C followed by Chlorpyrifos and Proclaim with percent lethality of 80%. However, statistical analysis revealed no significant differences among these treatments. Lowest percent mortality of 46.66% was induced by Karate and Match after 24 h. After 48 h, complete mortality (100%) was achieved in Polytrin-C and Proclaim followed by Chlorpyrifos and Karate with mean percentage of 86.66% and 66.66% respectively, whereas in case of Match mean percent mortality was increased to 53.33%. After 72 h, percent mortality increased to 80% in Karate whereas no further increase was recorded in Chlorpyrifos and Match treatments.

The overall mean (average of 24, 48 and 72 h) of percent mortality exhibited that topmost mortality (97.77%) was recorded at recommended amount of Polytrin-C followed by Proclaim and Chlorpyrifos with percentage of 93.33% and 84.44%, respectively. However, Karate and Match caused 64.44% and 51.11% lethality, respectively and have minimal impact on pink bollworm larvae at their recommended quantity (Figure 1).

Percent mortality at low doses

The substantial toxicity differences were recorded between the tested chemicals after exposure to 24, 48, and 72 h at their low doses (Table 3). After exposure of 24 h, markedly high mortality was observed for Polytrin-C (53.33%) followed by Proclaim and Chlorpyrifos exhibiting 46.66% and 33.33% mortality, respectively. After 48 h of exposure, increase in percent mortality was recorded in all the treatments with maximum percent mortality of 73.33% in Polytrin-C followed by Proclaim (66.66%). However, Chlorpyrifos and Karate caused similar percent mortality of 60% after 48 hours whereas lowest percent mortality (20%) was observed in Match. After 72 h of exposure further increase in mortality was noted in all four treatments as 73.33% for Chlorpyrifos and Proclaim, 66.66% for Karate, and 53.33% for Match.

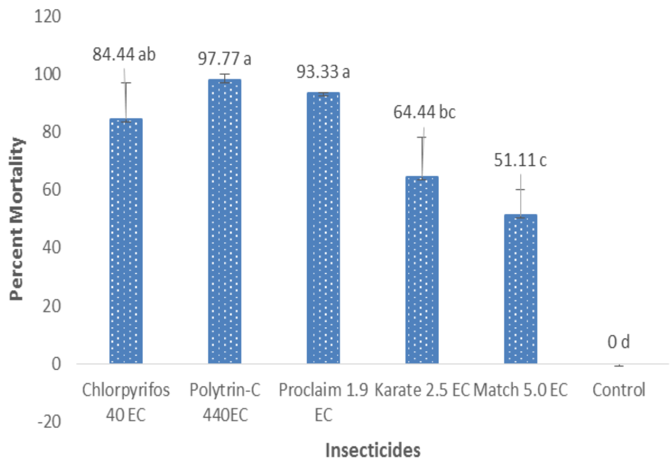

Figure 1: Overall mean (average of 24, 48 and 72 h) percent lethality of pink bollworm after exposure to various insecticides at recommended doses.

The overall mean (average of 24, 48 and 72 h) of percent mortality at low dose rates showed that Polytrin-C was the best amongst the tested ones against pink bollworm by inducing mortality of 66.66% followed by Proclaim (62.22%), Chlorpyrifos (55.55%) and Karate (51.11%). However, Match produced minimal impact at low dose amount by displaying 24.44% lethality (Figure 2).

Table 2: Effect of recommended doses of insecticides on the larvae of pink bollworm after different time intervals.

Treatments	Insecticides	Percent mortality (Mean ± S.E)		
		After 24 h	After 48 h	After 72 h
T1	Chlorpyrifos 40 EC	80 ± 11.54 a	86.66 ± 13.33 ab	86.66 ± 13.33 a
T2	Polytrin-C 440 EC	93.33 ± 6.66 a	100 ± 0.00 a	100 ± 0.00 a
T3	Proclaim 1.9 EC	80 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
T4	Karate 2.5 EC	46.66 ± 13.33 b	66.66 ± 17.63 bc	80 ± 11.54 a
T5	Match 5.0 EC	46.66 ± 13.33 b	53.33 ± 6.66 c	53.33 ± 6.66 b
T6	Control	0 ± 0.00 c	0 ± 0.00 d	0 ± 0.00 c

Mean sharing similar letters in columns are not significantly different.

Table 3: Effect of low doses (half of recommended) of insecticides on the larvae of pink bollworm after different time intervals.

Treatments	Insecticides	Percent mortality (Mean ± S.E)		
		After 24 h	After 48 h	After 72 h
T1	Chlorpyrifos 40 EC	33.33 ± 13.33 a	60 ± 11.54 ab	73.33 ± 13.33 a
T2	Polytrin-C 440 EC	53.33 ± 6.66 a	73.33 ± 13.33 a	73.33 ± 13.33 a
T3	Proclaim 1.9 EC	46.66 ± 6.66 a	66.66 ± 6.66 a	73.33 ± 13.33 a
T4	Karate 2.5 EC	26.66 ± 17.63 ab	60 ± 23.09 ab	66.66 ± 17.63 a
T5	Match 5.0 EC	0 ± 0.00 b	20 ± 11.54 bc	53.33 ± 13.33 a
T6	Control	0 ± 0.00 b	0 ± 0.00 c	0 ± 0.00 b

Mean sharing similar letters in columns are not significantly different.

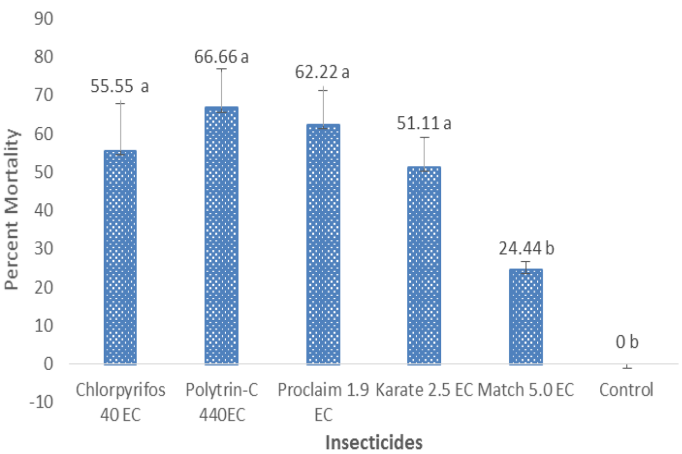

Figure 2: Overall mean (average of 24, 48 and 72 h) percent lethality of pink bollworm after exposure to various insecticides at low doses.

Discussion

Pink bollworm has an internal feeding habit; therefore, the timing of pesticide application is highly significant for its successful control. This is due to the complete development of larval duration within the bolls and after entering the bolls, the insecticide sprays become ineffective (Fand *et al*., 2020, 2021). The present findings reflected that all of the evaluated insecticides were proved efficient and led to substantial lethality of 3rd larval instar of pink bollworm. Furthermore, selection of compounds against pink bollworm in the current study was suitable as previously they were found to be efficient ones in managing cotton bollworms, e.g., chlorpyrifos against Earis spp. and pink bollworm (Dhawan *et al*., 1989); cypermethrin against pink bollworm (Swamy *et al*., 2000); lambda-cyhalothirn against cotton bollworms (Patel, 2013); emamectin benzoate against spotted bollworm and pink bollworm (Saleh *et al*., 2013); profenophos, cypermethrin, chlorpyrifos and lambda-cyhaothirn against pink bollworm (Zaki *et al*., 2015; Moustafa *et al*., 2019).

The results revealed that Polytrin-C was the topmost by exhibiting highest lethality followed by Proclaim and Chlorpyrifos at their field recommended quantity. Polytrin-C is the mixture of cypermethrin and profenofos. Cypermethrin is associated with synthetic pyrethroid class II and passes the blood-brain barrier and brings neurotoxicity in the CNS and also leads to motor loss. (Eells *et al*., 1988; Narahashi *et al*., 1992; Kirby *et al*., 1999). Profenofos belongs to organophosphates, acts as a nerve poison for insects, paralyzing and killing them. It binds to the acetyl-cholinesterase receptor once absorbed or ingested by the organisms that decreases the function of AChE receptor (Kushwaha *et al*., 2016).

In general, the products having multiple compounds are anticipated to be more effective as compared to when used alone for insect control at their field recommended quantity (European Plant Protection Organization, 2012). These findings are highly in accordance with the work of Busnoor *et al*. (2024) who recorded highly significant mortality of pink bollworm larvae after treating the boll rind with profenofos+cypermethrin, chlorpyrifos +

cypermethrin and lambda-cyhalothrin prior to the release of larvae. Similarly Naik *et al*. (2023) also reported that combined formulation of profenofos + cypermethrin proved better in reducing the larvae of pink bollworm, mines, exit holes and damage to locules in cotton. Zidan *et al*. (2012) showed excellent results of alpha-cypermethrin and lambda-cyhalothrin against pink bollworm. More *et al*. (2023) reported that Profenofos 50% EC showed high efficacy against pink bollworm and recommended for its better management. Similarly, bifenthrin 10 EC, cypermethrin 5% EC and lambda cyhalothirn 2.5 EC gave effective control in decreasing the bollworms population (Khan *et al*., 2007; Balakrishman *et al*., 2009). El-Basyouni (2003) reported that insecticides belong to synthetic pyrethroids were more efficient than compounds belonging to carbamates and organophosphate insecticides. Hnialum *et al*. (2022) showed superior potency of Profenofos 50 EC and Cypermethrin 25 EC as ovicide against the pink bollworm eggs.

The present study has indicated that Proclaim 1.9 EC containing emamectin benzoate as active ingredient was the second best insecticide bringing about uppermost percent mortality of pink bollworm larvae. Emamectin benzoate is associated with 2nd generation of avermectins possessing distinct toxic capability against the pests belonging to order lepidoptera (Vargas *et al*., 1997). It activates chloride channels by lessening the excitability of neurons. Following exposure, feeding ceases and larvae die due to irreversible paralysis (Cardwell *et al*., 2005). Likewise, Busnoor *et al*. (2024) reported 99.98% mortality of pink bollworm larvae after treatment of green bolls before the release of larvae with Emamectin benzoate 5 % SG under lab conditions. Similarly, Mouharib (2009) and Abbas *et al*. (2017) also observed superior performance of emamectin benzoate by giving maximum lethality of pink bollworm.

After Proclaim, Chlorpyrifos showed better toxic potential whereas Karate (lambda-cyhalothrin) and Match (leufenuron) were least effective ones against the pink bollworm larvae at their recommended doses. Chlorpyrifos is the organophosphate insecticide while Karate and Match belongs to pyrethroid and benzoylurea groups, respectively. These results are in agreement with the study of Rajput *et al*. (2017) who found Chlorpyrifos as most efficient insecticide against pink bollworm. In contrast, Ahmed (2020) noted superior potency of lambda-cyhalothrin over emamectin benzoate against 1st instar larvae of pink bollworm in laboratory.

Conclusion

It could be interpreted from the current findings that Polytrin-C 440 EC (profenofos + cypermethrin) and Proclaim 1.9 EC (emamectin benzoate) might be the first option for the control of pink bollworm in cotton. However, repeated usage of similar insecticides speed up the process of resistance development. To hinder this, insecticides belonging to different groups and having different mode of action should be rotated and frequent applications beyond recommended doses should be avoided. As the present results from laboratory trial are promising, further validation from field trails are suggested for future studies.

Declarations

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IRB approval

This study was approved by the Institutional Review Committee of Nuclear Institute of Agriculture, Tando-jam-70060, Pakistan.

Ethical statement

We ensured that all research was conducted in accordance with ethical principles. Neither human was the subject in research nor such kind of animal, which required any administrative approval.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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