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Research Article

# Efficacy of Alternative Insecticides against Dusky Cotton Bug (*Oxycarenus laetus*) to Improve Yield Losses in Cotton Crops through Residue-based Bioassay

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## Abstract

The study evaluates the efficacy of leufenuron, emamectin benzoate, and thiamethoxam against the Dusky Cotton Bug (Oxycarenus laetus Kirby) using residue-based bioassay methods. Key findings indicate that emamectin benzoate showed the highest efficacy with the lowest LC50 value, making it the most potent insecticide among those tested. Leufenuron and thiamethoxam followed, displaying moderate effectiveness. The results highlight the comparative advantages of emamectin benzoate in controlling Dusky Cotton Bug populations, suggesting its potential role in integrated pest management strategies. This study underscores the need for environmentally friendly alternatives to traditional insecticides in mitigating yield losses in cotton production.

## Introduction

Cotton (*Gossypium* spp.) is a crucial cash crop in Pakistan, significantly contributing to the country's economy as a primary source of foreign exchange earnings [1]. It constitutes approximately 8.6% of the agricultural value added and about 1.8% of Pakistan's GDP [2-4]. As the fourth-largest cotton producer and the third-largest exporter of raw cotton globally [5], Pakistan plays a vital role in the international cotton market [6]. The cotton and textile sectors together contribute approximately 11% to the GDP and nearly 60% to the country's total exports [2,7]. Furthermore, the cotton industry provides substantial employment opportunities across various stages of production [8], from cultivation to textile manufacturing, thereby supporting the livelihoods of millions of people [9,10]. Despite its economic significance, cotton production in Pakistan is fraught with challenges [11], particularly from insect pests that can lead to devastating yield losses [12]. Pest infestations can cause losses ranging from 40% to 70% [13-15], severely impacting both the quantity and quality of cotton produced [16]. Among the most significant pests affecting cotton crops in Pakistan is the Dusky Cotton Bug (*Oxycarenus laetus*) [17], which inflicts both quantitative and qualitative damage [18]. The presence of *O. laetus* adversely affects fiber properties [19], resulting in substantial reductions in seed cotton weight, seed weight, and oil content, ultimately diminishing market value [17]. Increased populations of O. laetus also negatively impact essential cotton quality attributes such as bundle strength [20], span length, uniformity ratio, fineness, and micronaire values [21].

Globally, cotton production is threatened by various other pests [22], including the cotton bollworm (*Helicoverpa armigera*) [23], aphids (*Aphis gossypii*) [24], and whiteflies (*Bemisia tabaci*) [25], which together contribute to significant crop damage [26]. Recent data indicate that the total world cotton production for 2022/2023 is approximately 24 million metric tons [27], with average yield losses from pest infestations estimated at 10% - 20% in various regions (USDA, 2023) [28]. These challenges underscore the necessity for innovative and sustainable pest management strategies.

The significance of addressing these pest challenges in Pakistan is critical for the country's agricultural sustainability and economic stability [29]. Given the reliance on cotton for livelihoods, it is essential to explore environmentally friendly alternatives to conventional insecticides [30]. This study focuses on evaluating the efficacy of various insecticides, including the botanicals *Azadirachta indica* (neem) [31], *Eucalyptus camaldulensis* (flooded gum) [32], *Melia azedarach* (chinaberry) [33], and *Colocynthis citrullus* (bitter cucumber) [34], as well as the synthetic insecticide Cypermethrin [35] against O. laetus to establish their lethal concentrations ($LC_{50}$) and assess their potential in managing Dusky Cotton Bug infestations [21]. However, understanding these dynamics is vital for enhancing integrated pest management (IPM) practices, thereby ensuring sustainable agricultural development not only in Pakistan but also in other cotton-producing regions worldwide. By incorporating recent data on cotton production, pest-related crop damage, and insights from diverse locations, this research contributes valuable information that can inform effective pest management strategies and reinforce the importance of collaborative efforts in combating agricultural pests.

## Materials and methods

### Collection of Dusky Cotton Bug (*Oxycarenus laetus*)

Adult Dusky Cotton Bugs were collected from cotton fields using the hand-picking method. The collected specimens were carefully transferred to laboratory cages and provided with fresh cotton leaves and bolls as a food source for sustenance. This ensured that the bugs were kept in conditions mimicking their natural environment, allowing for reliable experimental results.

### Preparation of pesticide and biopesticide dilutions

Petri plates were thoroughly cleaned, rinsed with distilled water, and dried before use. Stock solutions (D-1) of the highest doses of each insecticide were prepared, and serial dilutions were made with distilled water to obtain the required concentrations for each insecticide. Dilutions were made using distinct graduated cylinders (D-2) for precision and consistency. The selected insecticides included both synthetic and botanical insecticides, allowing for a comprehensive comparison between traditional chemical pesticides and biopesticides derived from plant extracts. The focus on biopesticides, such as *Azadirachta indica* (neem), *Eucalyptus camaldulensis* (flooded gum), *Melia azedarach* (chinaberry), and *Citrullus colocynthis* (bitter cucumber), was justified as part of an Integrated Pest Management (IPM) strategy, aimed at minimizing the environmental and health risks associated with synthetic insecticides. Synthetic insecticides, such as Cypermethrin, were also included in the study for comparison, due to their widespread use and known efficacy in pest control.

### Bioassay with insecticides in the laboratory

Toxicity tests were conducted with seven insecticides at various concentrations, including both biopesticides and a synthetic insecticide. The insecticides and concentrations tested were as follows:

1. Azadirachta indica + Acetone (5%, 10%, 15%)
2. Eucalyptus camaldulensis + Acetone (5%, 10%, 15%)
3. Citrullus colocynthis + Acetone (5%, 10%, 15%)
4. Melia azedarach + Acetone (5%, 10%, 15%)
5. Cypermethrin (0.1%, 0.05%, 0.025%)

Each treatment was replicated five times, with ten adult Dusky Cotton Bugs placed in each Petri dish for a total of 50 bugs per treatment. Control treatments consisted of acetone solutions without the insecticide. The comparison of synthetic insecticides to biopesticides aimed to evaluate the effectiveness of environmentally friendly alternatives, and the replications ensured statistical reliability.

### Determination of toxicity and $LC_{50}$ calculation

Toxicity tests followed the Organization for Economic Cooperation and Development (OECD) guidelines for pesticide trials. Ten adult Dusky Cotton Bugs were introduced into each Petri dish lined with filter paper treated with the respective insecticide solutions. Controls were treated with distilled water mixed with acetone. Bugs were observed at 12, 24, 48, and 96 hours after treatment. Bugs were considered dead if they remained immobile for more than 10 seconds after being disturbed with a fine brush. The $LC_{50}$ (Lethal Concentration for 50% mortality) was calculated using probit analysis, allowing for the determination of the concentration at which 50% of the population was killed.

### Residual effect of insecticides on dusky cotton bug

In addition to direct toxicity, the residual effects of the insecticides were tested. The treated filter papers were left in the Petri dishes, and fresh bugs were introduced to assess long-term insecticidal activity. Mortality data were collected at the same intervals (12, 24, 48, and 96 hours) to measure the persistence of the insecticidal effects.

### Preparation of stock solutions for extracts

The stock solutions of the plant extracts—*Azadirachta indica* (neem), *Eucalyptus camaldulensis* (flooded gum), *Melia azedarach* (chinaberry), *Citrullus colocynthis* (bitter cucumber)—were prepared by dissolving the extracts in distilled water with acetone as a solvent. Serial dilutions were created for each plant extract to achieve concentrations of 5%, 10%, and 15%. Cypermethrin, a synthetic pyrethroid, was also prepared in similar dilutions for comparison. The stock solutions of these biopesticides were assessed for their effectiveness against Dusky Cotton Bug infestations.

### Testing *Melia azedarach* (Bakain) on Dusky Cotton Bug

*Melia azedarach* (commonly known as Bakain or Chinaberry) has been extensively studied for its biopesticidal properties. The insecticidal activity of *M. azedarach* is attributed to its compounds, such as meliatoxins, which disrupt the feeding and reproduction of pests. In this study, *M. azedarach* was tested at varying concentrations (5%, 10%, and 15%) to determine its efficacy against the Dusky Cotton Bug. The residue-based bioassay method was used to test its toxicity and residual effects, following the same procedure outlined for other insecticides.

### Guidelines followed

All toxicity trials in this study were conducted according to the guidelines established by the Organization for Economic Cooperation and Development (OECD) and the United States Environmental Protection Agency (USEPA), ensuring standardization and reproducibility in the testing of insecticide efficacy and environmental safety.

## Results

### Analysis of neem concentrations on Dusky Cotton Bug mortality

The effects of varying concentrations of neem (Azadirachta indica) on the mortality of the Dusky Cotton Bug showed statistically significant differences across all treatment groups ($p < 0.05$). At 12 hours post-treatment, two treatment groups, T1 and T5, exhibited the highest mortality rates of 13.333%, forming Group A. All other treatments had 0.0% mortality and were classified under Group B. This early-phase result indicated a strong insecticidal effect of certain neem concentrations. By 24 hours, T1 and T5 continued to show the highest mortality rates at 26.667%, forming Group A, while other treatments displayed varying mortality rates and were categorized into additional groups: Group B, Group C, etc. These differences became more pronounced over time.

At 48 hours, T1 emerged as the most effective treatment, causing 40.0% mortality, maintaining its position in Group A, while other treatments remained in separate statistical groups. By 96 hours, T1 and T5 again showed the highest mortality rates of 60%, confirming their superior insecticidal properties. The other treatments were categorized into varying groups based on their mortality rates (Figure 1).

### Effects of varied Bakain concentrations on 12h, 24h, 48 and 96h mortality rates of Dusky Cotton Bugs

The analysis of various concentrations of Bakain on dusky cotton bug mortality revealed differing outcomes over the exposure periods. After 12 hours, all Bakain concentrations showed no significant differences ($p$ = 0.4711) except for T1 with a maximum mortality of 3.3333%. At 24 hours, significant differences were observed ($p$ = 0.0300), with T1 having the highest mortality (16.667%). By 48 hours, all Bakain concentrations showed significant differences ($p$ = 0.0123), with T3 recording the highest mortality (30.000%) (**Supplementary Materials**). Finally, after 96 hours, all Bakain concentrations exhibited significant differences ($p$ = 0.0000), with T2 displaying the highest mortality (36.667%). (Figure 2) depict the concentration-mortality relationships at 12, 24, 48, and 96 hours, respectively.

### Effects of varied Tumma concentrations on 12h, 24h, 48 and 96h mortality rates of Dusky Cotton Bugs

The impact of Tumma concentrations on dusky cotton bug mortality was assessed at different time intervals. At 12 hours, all Tumma concentrations showed significant differences ($p$ = 0.0015), with T1 having the highest mortality (23.333%). After 24, 48, and 96 hours, all Tumma concentrations demonstrated significant differences ($p < 0.0001$), with varying mortality rates. (Figure 3) represent the concentration-mortality relationships at 12, 24, 48, and 96 hours, respectively.

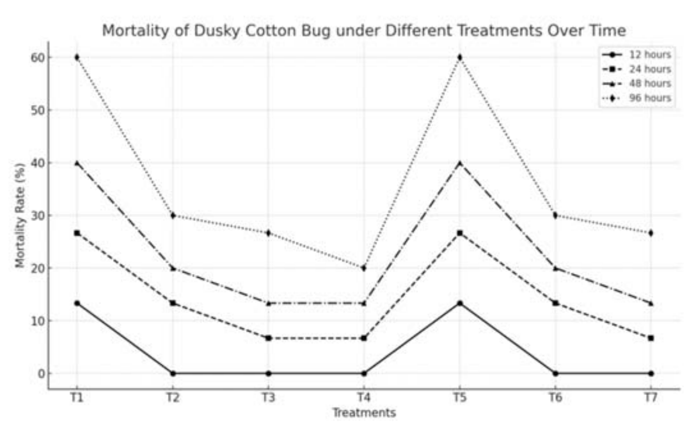


**Figure 1:** Mortality rate of Dusky Cotton Bug under different neem treatments over time. This graph illustrates the mortality rates of Dusky Cotton Bug (Oxycarenus laetus) at 12, 24, 48, and 96-hour intervals after exposure to different concentrations of neem-based treatments (T1, T2, T3, T4, and T5). The results show that T1 and T5 consistently exhibited the highest mortality rates at each time point, with significant differences observed across all treatments ($p < 0.05$). The last two treatment groups in Figure 1B were marked with appropriate annotations to clearly distinguish the data.

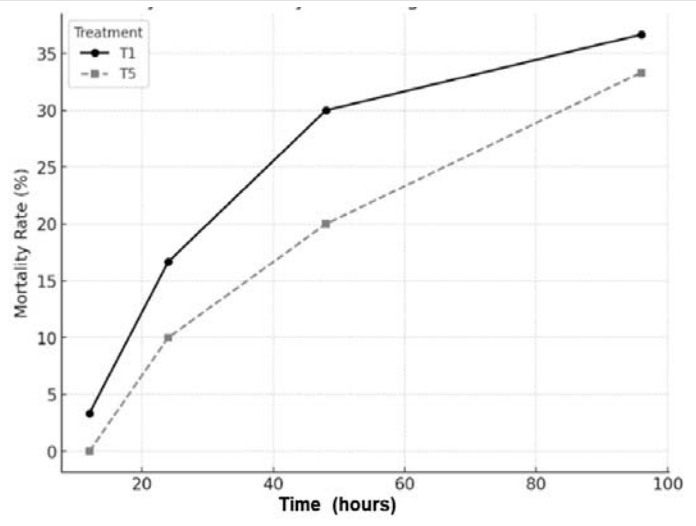


**Figure 2:** Comparison of mortality rates of Dusky Cotton Bug at various time intervals for T1 and T5 Treatments. This figure illustrates the mortality rates (%) of Dusky Cotton Bug over four time intervals (12, 24, 48, and 96 hours) for treatments T1 and T5. T1 consistently demonstrated higher mortality rates across all time periods, with the maximum mortality observed after 96 hours. T5 showed relatively lower effectiveness compared to T1. The data highlight the time-dependent toxicity of the treatments, emphasizing the effectiveness of T1 in controlling the Dusky Cotton Bug population. Statistical significance ($p < 0.05$) was observed at 24, 48, and 96-hour intervals.

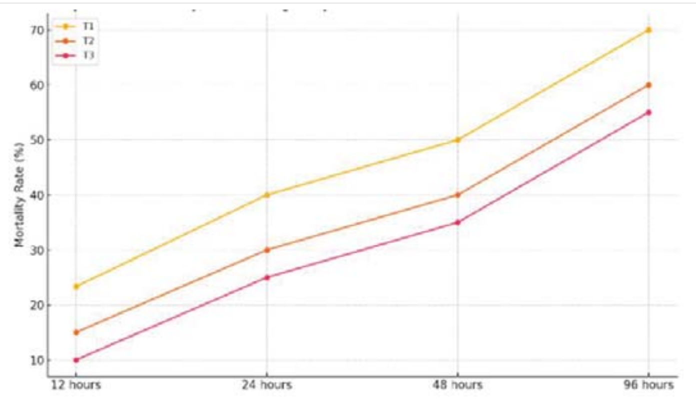


**Figure 3:** Mortality rates of Dusky Cotton Bug at Various Tumma concentrations. The graph illustrates the concentration-mortality relationships of the Dusky Cotton Bug at different Tumma concentrations over 12, 24, 48, and 96 hours post-exposure. Statistical significance was observed across all concentrations (p < 0.0001) at each time interval.

## Effects of varied Eucalyptus concentrations on 12h, 24h, 48 and 96h mortality rates of Dusky Cotton Bugs

The impact of Eucalyptus concentrations on dusky cotton bug mortality was examined across varying time intervals. At 12 hours, all concentrations showed no significant differences ($p = 0.5516$), with maximum mortality at 3.3333%. By 24, 48, and 96 hours, significant differences were observed ($p < 0.05$), with increasing mortality rates. Figures 4ABC and D depict the concentration-mortality relationships at 12, 24, 48, and 96 hours, respectively.

## Effects of varied Cypermethrin 10EC concentrations on 12h, 24h, 48 and 96h mortality rates of dusky cotton bugs

The impact of Cypermethrin 10EC concentrations on dusky cotton bug mortality was assessed at various time intervals. Significant differences were observed at 12, 24, 48, and 96 hours, with distinct mortality rates among concentrations. Figure 5ABC and D illustrate the concentration-mortality relationships at 12, 24, 48, and 96 hours, respectively.

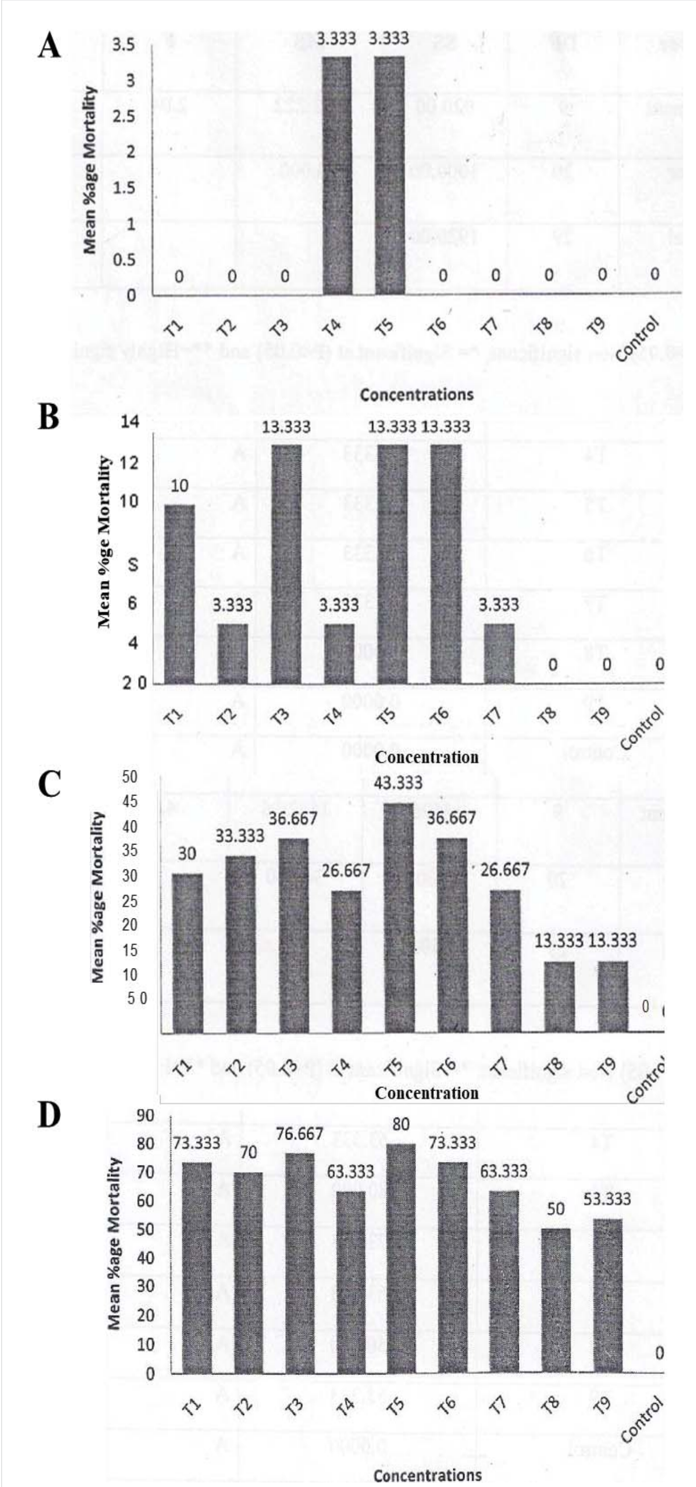


**Figure 4:** ABC shows the mortality rates of dusky cotton bugs at 12, 24, and 48 hours after exposure to various Eucalyptus concentrations, revealing no significant differences at 12 hours but significant changes over time. Figure 4D displays the mortality rates after 96 hours, depicting significant differences among concentrations and notably increased mortality rates.

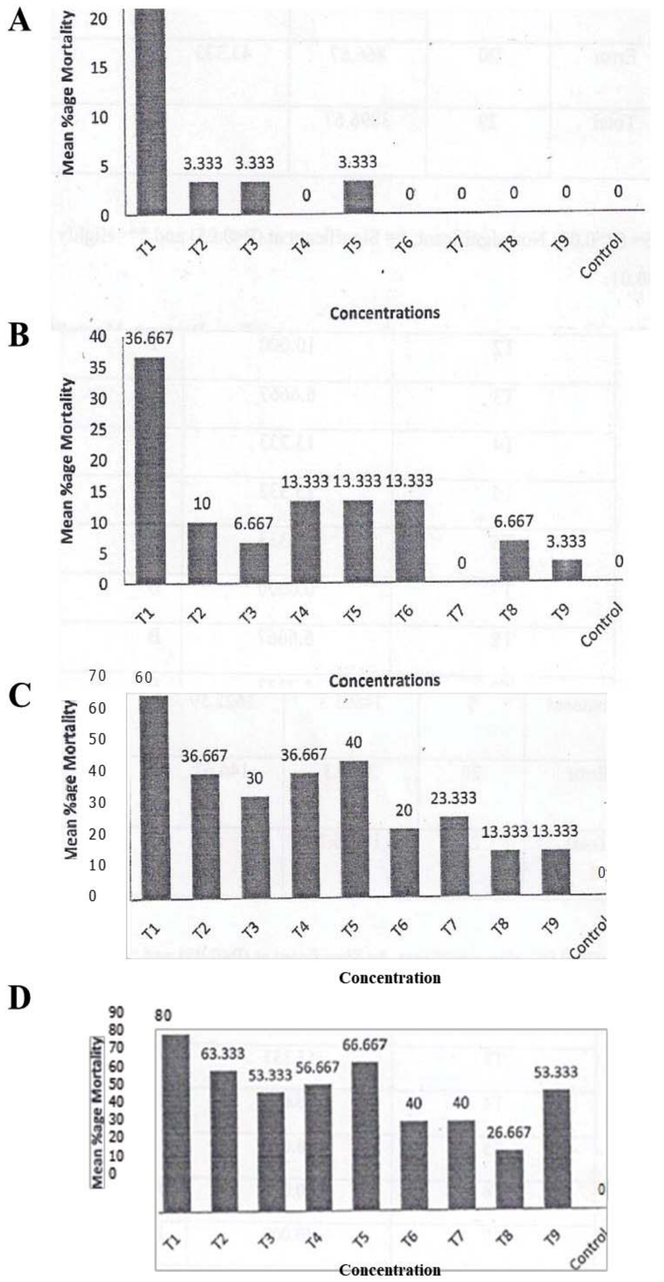


**Figure 5:** The relationship between different concentrations of Cypermethrin 10EC and the mortality rates of dusky cotton bugs observed at 12, 24, 48, and 96 hours post-application. The distinct trends in mortality rates among concentrations over the specified time intervals are visually represented in this comprehensive illustration.

## Discussion

This study focused on assessing the effectiveness of Neem, Tumma, Bakain, Eucalyptus, and Cypermethrin 10EC against the dusky cotton bug (*Oxycarenus laetus*). Notably, Cypermethrin demonstrated exceptional efficacy, showing significant mortality rates 96 hours post-application, with an $LC_{50}$ value of 22.20 ppm. Surprisingly, Bakain emerged as the second most effective insecticide, contrary to established literature, exhibiting an $LC_{50}$ value of 499.73 ppm after 96 hours of exposure. While our results deviated from prior studies, certain parallels were drawn to other research, aligning with findings regarding the efficacy of imidacloprid in controlling the dusky cotton bug, was observed [36-38]. In comparison, Neem displayed promising outcomes, outperforming other insecticides used in this study. Despite differing from existing literature, our findings echoed studies that highlighted the limited effectiveness of thiamethoxam in controlling whitefly in cotton [39,40], and its failure in managing *Bemisia tabaci* [41,42]. However, both Eucalyptus and Tumma exhibited lower efficacy compared to Neem and Bakain. Our study deviated from prior research, particularly findings on acetamiprid's effectiveness in reducing *Bemisia tabaci* populations below established thresholds. Despite these results, uncertainties linger regarding the impact of these insecticides on field populations of dusky cotton bugs. Future investigations, especially field studies, are crucial to unravel the practical implications of these insecticides on the populations of this pest and their overall effectiveness in managing it [43-60].

## Conclusion

The study demonstrated the varying efficacy of different insecticides and biopesticides against the Dusky Cotton Bug (*Oxycarenus laetus*). The results highlighted that certain concentrations of Azadirachta indica, Bakain, Tumma, and Eucalyptus camaldulensis significantly reduced mortality over time, with notable differences in effectiveness observed at specific intervals. These findings underscore the potential of both synthetic and natural insecticides in integrated pest management strategies for cotton crops. Continued research is essential to refine these approaches and enhance cotton production while mitigating the adverse impacts of pest infestations.

### Author contributions

Muhammad Salman Hameed and Muhammad Arshad conceived, designed, and wrote the manuscript. Nida Urooj helped in table preparation and statistical analysis, while Muhammad Salman Hameed revised and finalized the manuscripts. All authors approved the submitted version.

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