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Effect of Insecticides on Predatory Efficiency of *Coccinella Septempunctata* in a Prey Plant System

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Abstract

The seven-spot ladybird (*Coccinella septempunctata*) is a key predator of aphids, whiteflies, leafhoppers, and mealybugs. However, the application of insecticides to control these pests can impair its predatory efficiency. This study evaluated the effects of four insecticides: imidacloprid, pyriproxyfen, abamectin, and acetamiprid on predatory feeding, aphid mortality, and foraging behavior of *C. septempunctata* in a treated cropping system, using a completely randomized sample contains five-replicate experimental design. The results showed that pyriproxyfen (T2) improved predatory feeding and foraging behavior in both males (26.6%) and females (29.6%), while imidacloprid (T1) resulted in the highest aphid mortality rate (67% in males, 71% in females). Pyriproxyfen also demonstrated superior predator-prey interaction. In contrast, imidacloprid proved most effective at controlling aphids and significantly reduced predation. The researchers found that pyriproxyfen was the most suitable insecticide for increasing the predatory efficiency of *C. septempunctata*, which exhibited the best performance in feeding and foraging, while imidacloprid resulted in the highest aphid mortality at the expense of predator activity. These results indicate that the compatibility of insecticides with natural predators is an essential aspect to consider for integrated pest management.

Keywords: *Coccinella septempunctata*; Insecticide compatibility; Predatory efficiency; Integrated Pest Management; Sublethal effects

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Introduction

Seven spotted ladybird beetle, *Coccinella septempunctata* (Coccinellidae: Coleoptera) is a common biological control agent that found in various agricultural crops (Harwood et al., 2007). These predatory beetles have 6,000 species in the world, and they are small, approximately 0.8 to 18 mm in length. They are yellow, orange or red in color with small black spots on their forewings (Che, L et al., 2021; Hodek et al., 2012). This insect predator is known for its feeding efficacy and controlled the population of different soft bodied insect pests such as aphids, whiteflies, leafhoppers, mealybugs and scale insects (Cicero et al., 2024). This predator has different feeding and developing periods because sometimes they feed the honeydew, pollen, plant sap, nectar and various fungi for their survival (Sarwar et al., 2010; Almeida et al., 2011).

The insecticides show the acute as well as sublethal impacts on the physiology and behavior of survived individuals. These impacts concern significant unprotected survived organisms regarding the population level (Desneux et al., 2007). These chemicals must be applied according to insect ETL; however, the selected substances and treatment techniques must be utilized to reduce the population of pests and prevent the population of predators. These are the basic elements of Integrated Pest Management (Naranjo and Ellsworth, 2009).

In IPM, the release of biocontrol agents as well as the spray of agro-chemicals is the major components that reduce the pest population. Therefore, when the pest population shows the low appearance, the natural enemies stable their activity, but the population raised at economic injury level (EIL), the insecticidal spray must be applied for the reduction (Luna et al., 2018). Various beneficial insects that are

found in the agro-ecosystem have been impacted by the extensive use of pesticides (Lu et al., 2012). The neonicotinoid agro-chemicals have least toxic to the birds and mammals and these are rapidly killed to the pest (Tomizawa and Casida, 2005).

The excessive use of chemical pesticides shows the negative impact and produces resistance in the pest, contaminating the agro-ecosystem and harmful for predators and parasitoids (Elzen, 2001). Broad-spectrum are the chemicals that suppress the highly damage of aphids in the field condition, but these

chemicals are significantly toxic to the beneficial organisms (Lu et al., 2012). The biocontrol agents living in the field atmosphere are mostly unprotected because the pesticides are applied on aphids and other insect pests by topical and foliar methods. However, these aphids as well as floral parts of plant are contaminated through chemical droplets and when the predators feed on the prey and the nectar of the plant; they are mostly damaged (Cabral et al., 2011). Among different insecticides, imidacloprid is a systemic poison which intake by the plant through their roots. Then roots transport the poison into vascular system where it effects on the plant feeding pests (Tomlin, 2006). It is an effective pest control poison that reduces the population of pests in a natural ecosystem (Matsuda et al., 2001). This poison effect as a nicotinic acetylcholine (Ach) agonist and combine snicotinergic receptors in the postsynaptic nerves and prevent the acetylcholine from the binding process (Epstein, M. 2020).

The main objective of this study is to systematically evaluate and rank four commonly used insecticides – imidacloprid (a neonicotinoid), pyriproxyfen (an insect growth regulator), abamectin (an avermectin), and

acetamiprid (a neonicotinoid) based on their compatibility with *C. septempunctata* and their impact on predation efficiency. We hypothesize that the mechanism of action and chemical class of each insecticide will differently influence predator consumption, aphid mortality, and the foraging behavior of *C. septempunctata*. Specifically, pyriproxyfen, as an insect growth regulator, is expected to exhibit greater predator compatibility, minimally interfering with foraging behavior and maintaining high predator consumption rates. Conversely, neuroactive insecticides such as imidacloprid are expected to significantly impair predator activity due to their neurotoxic effects. By quantifying consumption rates, aphid mortality, and foraging behavior in male and female beetles, this study aims to provide a comparative ranking of insecticide compatibility that will inform integrated pest management strategies designed to optimize both chemical pest control and the conservation of beneficial natural enemies.

Materials and Methods

Experimental Design and Study Site.

The experiment was performed in Biocontrol Research Laboratory, Department of Entomology, faculty of crop protection, Sindh Agriculture University, Tando jam, Pakistan. The experimental design adopted was a Complete Randomized Design (CRD) and five replications were used to provide credible findings.

The species and rearing environment of insects.

Aphids: *Aphis erysimi* (Kaltenbach) (Hemiptera: Aphididae), which is a widespread species on brinjal vegetation, was utilized in this research. Aphids were collected in the field as naturally infested plants and reared in the laboratory under the controlled conditions in brinjal plants (*Solanum melongena*). To avoid cross-

contamination of the treatments, the aphids were kept in different rearing cages.

Ladybird Beetles: *Coccinella septempunctata* (Linnaeus) (Coccinellidae: Coleoptera) is a predator species that was reared in the laboratory. The adults of the beetles were taken in the field and held in cages of rearing under the same conditions as aphids. These cages were given aphids to feed on and the predators were given the opportunity to multiply within the presence of high aphid population.

Environmental Conditions

The conditions in the laboratory were manipulated so that there was consistency throughout the study:

Temperature: $25 \pm 2^\circ\text{C}$

Relative Humidity (RH): $65 \pm 5\%$

Photoperiod: 14 hours light and 10 hours darkness (L:D: 14:10) to mimic the natural conditions and allow normal predator and prey behavior.

Treatments: There were four different insecticides including control (water) i.e., T₁: Imidacloprid (1.0 ml/500 ml water), T₂: Pyriproxyfen (2.5 ml/500 ml water), T₃: Abamectin (1.0 ml/ 500 ml water), T₄: Acetamiprid (1.0 ml/500 ml water) and T₅: Control (500 ml water).

Procedure of experiment: Brinjal plants were grown into five plastic pods. 250 aphids (*Lipaphis erysimi*) were released on each plant. Foliar applications of each treatment were applied on brinjal plant. After application, each plant was kept 30 minutes for drying and released five each adult male and female of predator *C. septempunctata* for 24 h. and each plant was covered with plastic bag. The data on predation efficiency, mortality and foraging behavior of predator and prey was recorded after 2, 6 and 24 HAS (Hours After Spray). This experiment was designed to determine predation efficiency according to the model given by Soares et al. (2003).

$$(1) \quad V_0 = A - a_2$$

$$(2) \quad V_o = A - a_6$$

$$(3) \quad V_o = A - a_{24}$$

Whereas:

V_o = Number of eaten aphids

A = Number of aphids available

a_2, a_6, a_{24} = Number of aphids not consumed (dead or alive) in the system still 24 hrs. The consumption rate and mortality percentage of aphids were calculated by using the following formula:
Consumption rate % = $\frac{\text{consumed aphids}}{\text{Total aphids}} \times 100$

Total aphids = Mortality % = $\frac{\text{Dead aphid}}{\text{Total aphids}} \times 100$

Foraging behavior was assessed by recording the number of predators actively searching for prey on the plant surface at each observation time point

Statistical analysis: The data were subjected to statistical analysis using Statistix 8.1 computer software (Statistix, 2006). The differences among the treatment's means were compared by the LSD test, where necessary.

Results

Average consumption of *Coccinella septempunctata* males after the application of different insecticides at various intervals

Figure 1. The mean consumption rate of *Coccinella septempunctata* males on various time intervals (2, 6, and 24 hours after the spray). Pyriproxyfen (T2) was always the one with the highest predatory consumption reaching to the maximum of 26.6 aphids consumed at 24 hours, which is significantly higher than the rest of the treatments. T4 and T3 were also highly consumed by acetamiprid (T4), as well as, Abamectin. Imidacloprid (T1) was least consumed at all the time points, especially at 2 hours (7.2). The highest consumption of the control (T5) was at 24 hours (36.2 aphids). Standard error (SE) is represented by error bars. The pairwise comparison of ANOVA showed significant differences

($P < 0.05$) between treatments and time intervals.

Figure 1 Mean consumption of *Coccinella septempunctata* males after the application of different insecticides at various intervals. (Annexure A)

Mortality percentage of aphids in treated plants during predatory efficiency of *Coccinella septempunctata* males

Figure 2. The percentage of aphids killed when insecticides were applied to plants during the predatory efficiency of males of *Coccinella Septempunctata*. The y-axis indicates the percentage mortality of the aphids at different time durations (2, 6, and 24 hours) after the treatment with the insecticides. At 24 hours, the highest mortality rate of the aphids was observed with imidacloprid (T1, 67%), Abamectin (T3, 62%), Acetamiprid (T4, 46%) and pyriproxyfen (T2, 22%). Imidacloprid (31) was the deadliest at 6 hours and least active was Pyriproxyfen (18). Pyriproxyfen (13) recorded the highest mortality at 2 hours then Imidacloprid (20). The mortality was greatly reduced in the control (T5) at 24 and 2 and 6 hours, 8 and 4-7 percent, respectively. Standard error (SE) is represented by error bars. Basing on the pairwise comparisons of the ANOVA, there were notable differences ($P < 0.05$) between treatments and periods of time.

Figure 2 Mortality percentage of aphids in treated plants during predatory efficiency of *Coccinella septempunctata* males at various intervals (Annexure B)

Average consumption of *Coccinella septempunctata* females after the application of different insecticides at various intervals

Figure 3. Mean *Coccinella septempunctata* female consumption in different intervals. The y-axis was the mean number of aphids eaten at 2, 6 and 24 hours after spraying by the *C. septempunctata* females. Pyriproxyfen (T2)- was always the highest consumed at

all time and 29.6 aphids were consumed at 24 hours and at 6 hours; 24.8 aphids were consumed. At these time points, acetamiprid (T4), Abamectin (T3) and Imidacloprid (T1) had lower rates of consumption with the lowest rate of consumption being Imidacloprid (T1) at all the time intervals with the highest rate of consumption at 2 hours being 9.8 aphids. The highest total consumption was observed in the control (T5) at 24 hours (36.2 aphids), 2 and 6 hours (32.6 and 37.0 respectively). Standard error (SE) is represented as error bars. Based on ANOVA pairwise, significant differences ($P < 0.05$) were observed according to the treatment and time interval.

Figure 3 Mean consumption of *Coccinella septempunctata* females after the application of different insecticides at various intervals. (Annexure C)

Mortality percentage of aphids in treated plants during predatory efficiency of *Coccinella septempunctata* females

Figure 4. The percentage of aphid mortality of treated plants at different intervals during the predatory efficiency of *Coccinella septempunctata* females. The y-axis is a graph that gives the mortality rate of the aphids as a percentage. Error bars are the standard error (SE) of the mean and the letters above the bars show significant differences ($P < 0.05$) between the treatments and time points due to ANOVA pairwise results. Imidacloprid (T1) was found to be the most lethal at 24 hours after spraying, then Abamectin (T3) then Acetamiprid (T4) and finally, Pyriproxyfen (T2) recorded the lowest mortality. The control (T5) exhibited much lesser mortality than the treatment with insecticides.

Figure 4: Mortality percentage of aphids in treated plants during predatory efficiency of *Coccinella septempunctata* females at various intervals (Annexure D)

Foraging behavior of predator *Coccinella septempunctata* males on aphids in treated plant system at different intervals

Figure 5 indicates that Pyriproxyfen (T2) produced the highest overall consumption levels of *C. septempunctata* males 6 hours (21.0) and 24 hours (26.6) following spray, which means that it produced the most positive effect on the foraging behavior of the predator. This observation is in line with the mode of action of Pyriproxyfen as an insect growth regulator (IGR) that does not induce acute toxicity to adult predators and facilitates their predation. High consumption (17.8 and 22.0) was also promoted by acetamiprid (T4), and second by Abamectin (T3) (14.4 and 20.0), and Imidacloprid (T1) showed the least consumption (9.0 and 4.8), implying that its neurotoxic effects result in the inability of the predator to effectively forage.

The lowest consumption rate was recorded with Imidacloprid (7.2) at 2 hours after spray (HAS), which can be explained by the fact that it is a fast neurotoxicant against *C. septempunctata* that blocks movement and feeding behavior. The Pyriproxyfen (15.6) was the most consumed followed by Acetamiprid (12.2) and Abamectin (10.8) at this early stage, and that is why the Pyriproxyfen did not affect the behavior of the predator as much as the other insecticides.

The overall consumption rates (32.6 and 36.2) at 6 and 24 hours, respectively, were the highest in the control (T5) treatment, which proved that the presence of the insecticides had a significant negative impact on consumption in the treated plants.

Figure 5 Foraging behavior of predator *Coccinella septempunctata* males on aphids in treated plant system at different intervals (Annexure E)

Foraging behavior of predator *Coccinella septempunctata* females on aphids in treated plant system at different intervals

The rate of consumption was depicted by the difference in the *Coccinella septempunctata* females and the aphids in the treated plant systems as shown in figure 6. Pyriproxyfen (T2) was the most consumed at both 6 hours (24.8) and 24 hours (29.6) followed by Acetamiprid (T4), Abamectin (T3) and Imidacloprid (T1). Imidacloprid (T1) had the lowest consumption of 9.8 at 2 hours and at the same time, Pyriproxyfen had the largest consumption of 18.2 at 2 hours. The highest overall consumption was recorded in the control (T5) during the 6 and 24 hours (37.0 and 41.2, respectively) and this supports the effect of insecticides on predator foraging behavior. Statistical analysis showed that there were significant differences ($P < 0.05$) between treatments and time intervals.

Figure 6: Foraging behavior of predator *Coccinella septempunctata* females on aphids in treated plant system at different intervals (Annexure F)

Discussion

In the contemporary farming practice, natural predators can be inadvertently damaged due to the heavy application of chemical insecticides. Exposure of these predators to insecticides may be known to reduce its effectiveness as a biocontrol agent e.g. *Coccinella septempunctata*. Acute toxicity comes in as a direct effect and sublethal effects could be in the form of changes in feeding behavior, reproduction, predator-prey interactions. The need to minimize the impact of natural pest control as time progresses is of special concern to these effects.

The current research reveals how the usage of Pyriproxyfen, a growth regulator used on insects, keeps *C. septempunctata* predatoric efficiency intact. The selectivity

of Pyriproxyfen, which causes pests to molt is less toxic to adult predators and does not cause them much disturbance in their feeding or foraging habits. Integrated Pest Management (IPM) programs tend to use IGRs such as pyriproxyfen in preference due to their more ecologically friendly pest control method. The fact that pyriproxyfen had a minimal effect on the behavior of the predator in the current study is not new, as the IGRs have been found to have fewer sublethal effects on the beneficial arthropods than on the broad-spectrum insecticides (Abdel et al., 2016; Twardowski et al., 2021). The low toxicity of pyriproxyfen to predators may be explained by the limited systemic activity of this compound in non-target species and thus, enhances its compatibility with biocontrol agents considerably.

On one hand, Imidacloprid, a neonicotinoid insecticide, was found to be very toxic to *C. septempunctata*, especially on its foraging behavior. Imidacloprid as a neurotoxicant attaches to nicotinic acetylcholine receptors (nAChRs) in the nervous system of the insect paralyzing it and leading to its death (Tomizawa and Casida, 2003). This neurotoxic effect causing dramatic drop in predatory behavior of *C. septempunctata* is probably the reason why imidacloprid greatly reduces predatory behavior. It confirms previous researchers which have already shown the adverse effect of neonicotinoids on the behavior and physiology of ladybird beetles (Lucas et al., 2004; Skouras et al., 2019a, b). The negative impact of imidacloprid on the predator foraging indicates a significant drawback to the application of this insecticide in agro-ecology where biological management is a vital pest control method.

Although the action of Abamectin and Acetamiprid is not as toxic as that of

imidacloprid, they nevertheless had significant effect on *C. septempunctata*, but intermediate in relation to other insecticides. One example of *avermectins* is Abamectin, which is an *avermectin* that attaches to the glutamate-gated channels of chloride with the result of hyperpolarization and paralysis of the target pests (Eisenback et al., 2010). This action mechanism can be used as an explanation why the predation efficiency of this organism was lower in this experiment since the neurotoxic effects though not so strong as those of imidacloprid, still disturbed the foraging capacities of the predator. Likewise, Acetamiprid is not as damaging as imidacloprid, but it still resulted in pronounced decreasing the foraging behavior of *C. septempunctata*, which was presumably caused by its activity on *nAChRs*, albeit with a lower affinity to imidacloprid (Garzon et al., 2015). The findings of this paper point out that despite insecticides that are relatively less toxic to non-target organisms, sublethal effects can still lead to major changes in predator behavior, which ought to be taken into account during insecticides choice to control pests.

To sum up, the present study provides the important message of taking into account behavioral and physiological effects of the insecticides on the beneficial predators along with the effectiveness of the insecticides against the pests. Pyriproxyfen is the most appropriate insecticide to control the pests in systems that have natural predators such as *C. septempunctata* as part of pest control. IGRs are selective and minimum agents that influence the behavior of predators such that they could be an important part of IPM programs to achieve sustainable pest management. In contrast, Imidacloprid, though very effective in controlling aphid populations,

is very harmful on the populations of predators and should be used sparingly in a system where aphid populations are controlled by biocontrol techniques.

Conclusion

This paper showed that the predatory efficiency of male and female *Coccinella septempunctata* in the various insecticidal treatments exhibited significant differences which reflects the importance of the trade-off between the pests and natural enemies preservation. Although Pyriproxyfen (T2) was the least disruptive to predator behavior in promoting high rates of predatory feeding, Imidacloprid (T1) resulted into the highest number of aphids killed but it also led to large reduction in the foraging behavior of the predator. The findings indicate that all insecticides such as Pyriproxyfen, Abamectin, Acetamiprid, and Imidacloprid were useful in the elimination of aphids, but with different effects on *C. septempunctata*, with Imidacloprid and Abamectin being more toxic to the predator.

The results highlight the necessity to adopt an equal approach in Integrated Pest Management (IPM) programs, where selective application of insecticides is necessary to ensure that the negative impact on biocontrol agents, such as *C. septempunctata*, is reduced to a minimum. Although pest control is important to have healthy crops, it is also crucial to make sure that the usage of insecticides does not outcompete the functions of natural predators since they play an important role in the long-term pest control. As such we advise that since Pyriproxyfen is less toxic to natural enemies, it should be given a priority in pest control measures particularly in the areas where biological control agents are part of pest control.

Conclusively, the study has identified the importance of selective application of the insecticides in the IPM systems which has necessitated more sustainable methods of pest control that not only focus on immediate death of the pest but also the long-term protection of predators that are beneficial. With a combination of more insecticides that are less hostile to predators such as Pyriproxyfen the issue of pest reduction without compromising the effectiveness of natural predators can be achieved and eventually lead to a more sustainable and environmentally responsible way of dealing with pests.

Recommendations

There are a number of areas that should be addressed in future studies to enhance the findings of this study:

Sublethal Effects on Predator Reproduction and Larval Development: Although in this research article we were focused on the predatory consumption and foraging behavior of *Coccinella septempunctata*, this study did not exclude the importance of sublethal effects of insecticides on predator reproduction and larval development. To explore the long-term effect of insecticides and their impact on the natural population of ladybird beetles, the research into the influence of insecticides, and specifically neonicotinoids such as Imidacloprid and Acetamiprid, on their reproductive success and developmental stages will be more helpful.

Semi-Field and Field Trials: To confirm the laboratory results, semi-field and field trials will be done. Such experiments would give information about the behavior of insecticides in more natural environments, such as weather variations, dynamics of pests, and the existence of other biocontrol agents. The pesticides drift, residual toxicity and interaction effects would also be assessed in the field

since they are not entirely reproducible in the laboratory environment.

Residue Profiles on Plant Surfaces: This is a potential field of future study, the analysis of pesticide residue profiles on plant surfaces. The prediction of the long-term effects of the insecticides such as Imidacloprid, Abamectin and Pyriproxyfen on the foraging and reproduction of the predator would entail the information of the longevity of the harmful residues on plants. Remainder studies will facilitate the identification of the safe re-entry times of natural enemies and will yield very useful information to IPM programs as they can be able to know how to apply insecticides in a manner that would have minimal effects on biocontrol agents.

By covering these topics in the future, other studies can contribute to the improvement of our knowledge of the ecological effects of insecticides on natural predators and create more sustainable pest management methods.

Innovation

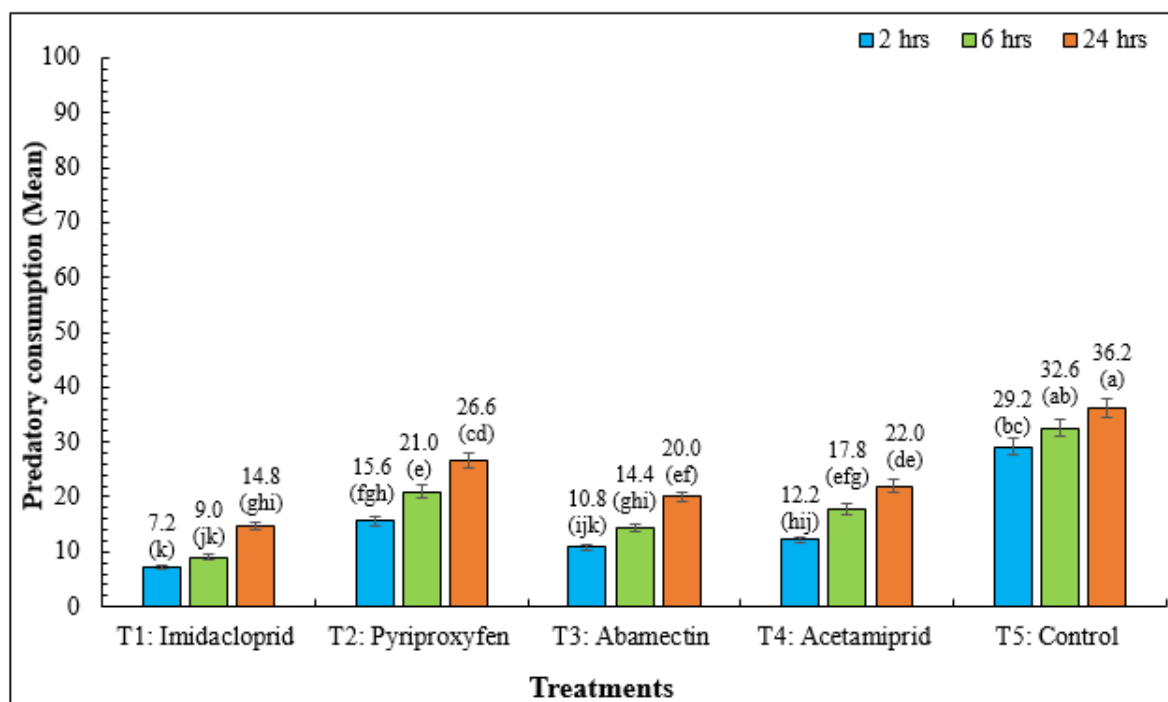
This paper is innovative and unique by making a direct comparison of insecticides with various modes of action with the predatory efficacy of *Coccinella septempunctata*, which is one of the essential biological controllers in the agricultural ecosystem. The empirical prioritization of insecticides, including Pyriprofen, Acetamiprid, Abamectin, and Imidacloprid, on the basis of their effect on predator feeding and foraging behavior gives key information on their useability in Integrated Pest Management (IPM) programs together with the natural enemies. This study does not just provide numbers to determine the impact of these insecticides in controlling the pests, but it also reveals the trade-offs between direct pest killing using insecticides and the preservation of predators that would be useful in pest control, thus putting

sustainable pest management approaches in a perspective of power. This research by providing evidence-based and clear recommendations on the use of selective insecticides will enhance the future of sustainable agriculture and support the idea of empowering humanity with knowledge through research that will develop more resilient and eco-friendly agricultural practices.

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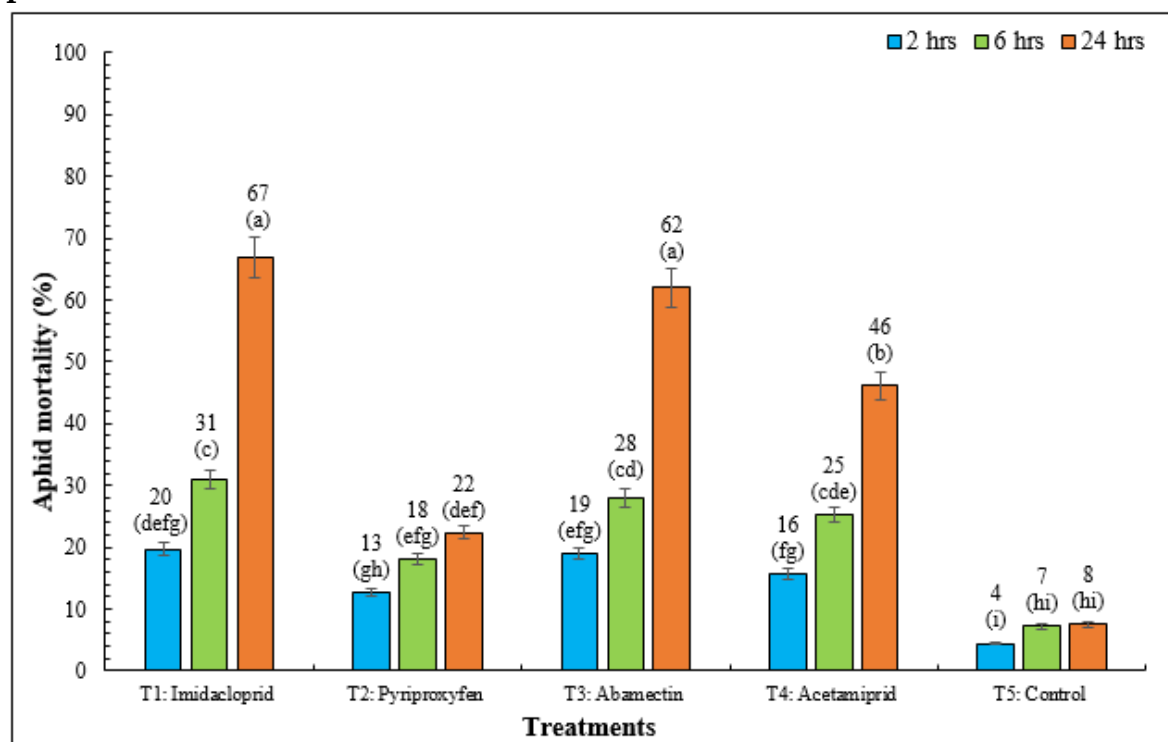
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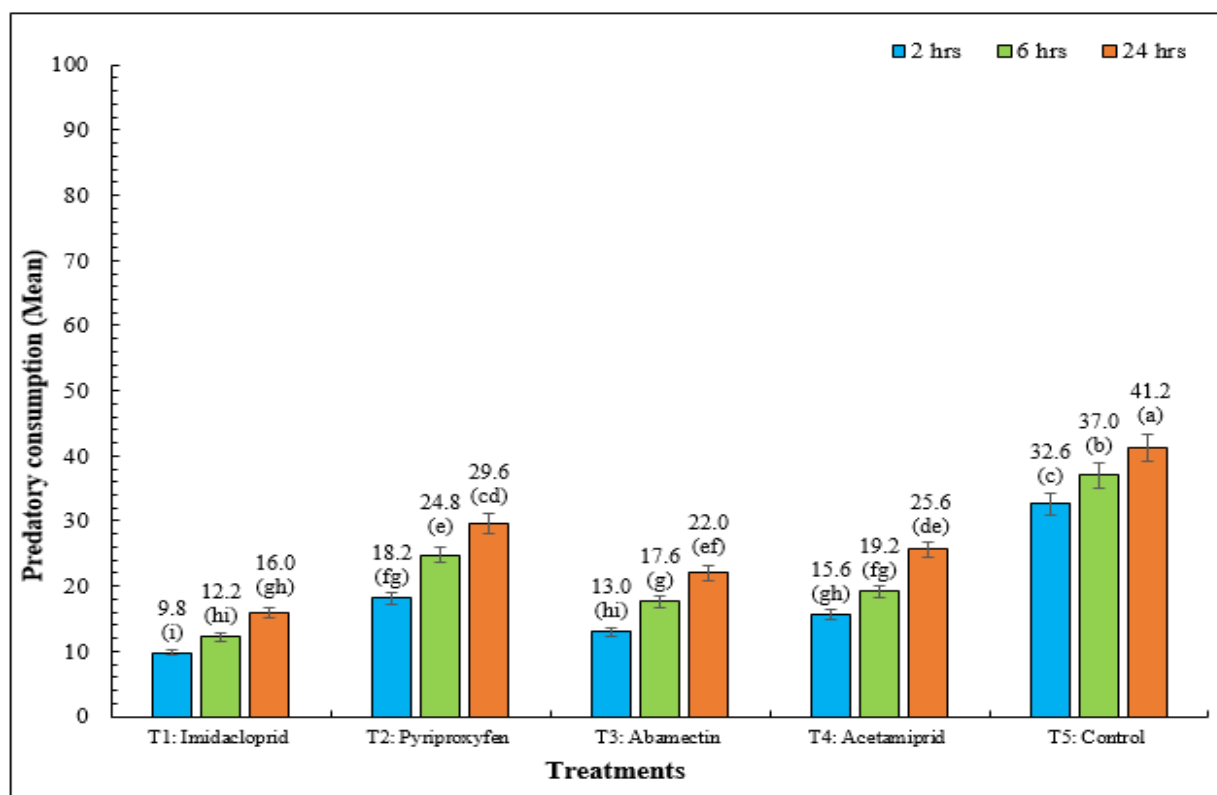
(Annexure A)

Figure 1 Mean consumption of *Coccinella septempunctata* males after the application of different insecticides at various intervals.



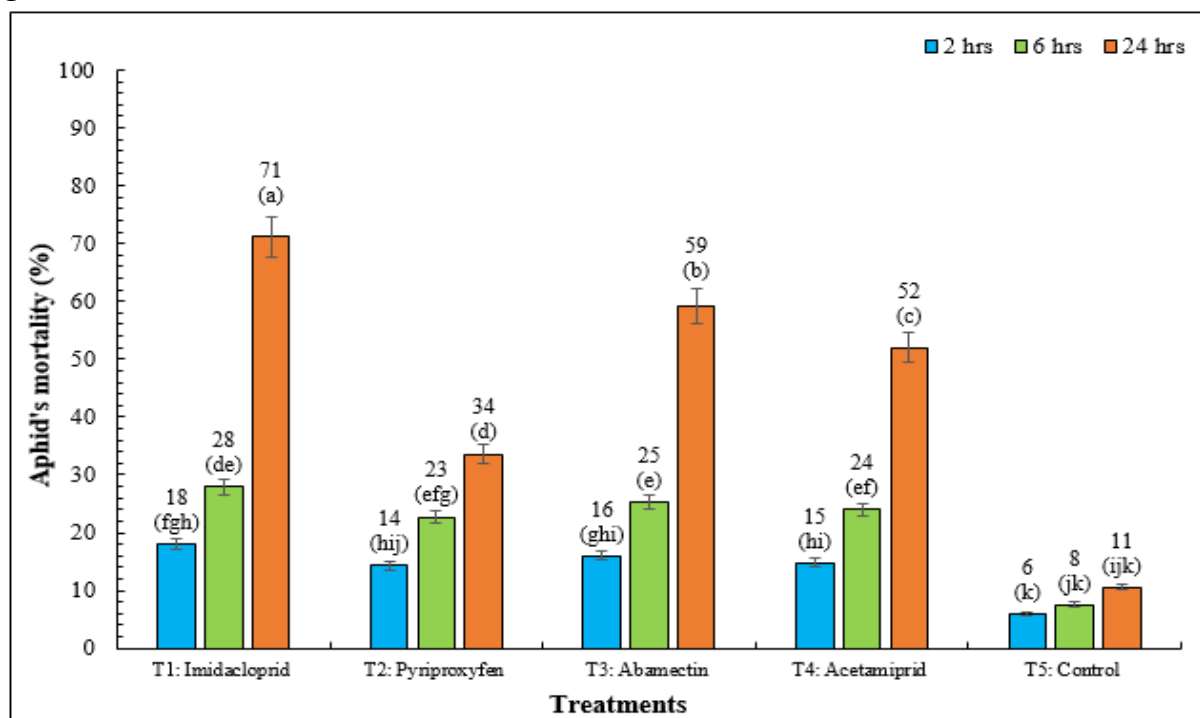
(Annexure B)

Figure 2 Mortality percentage of aphids in treated plants during predatory efficiency of *Coccinella septempunctata* males at various intervals



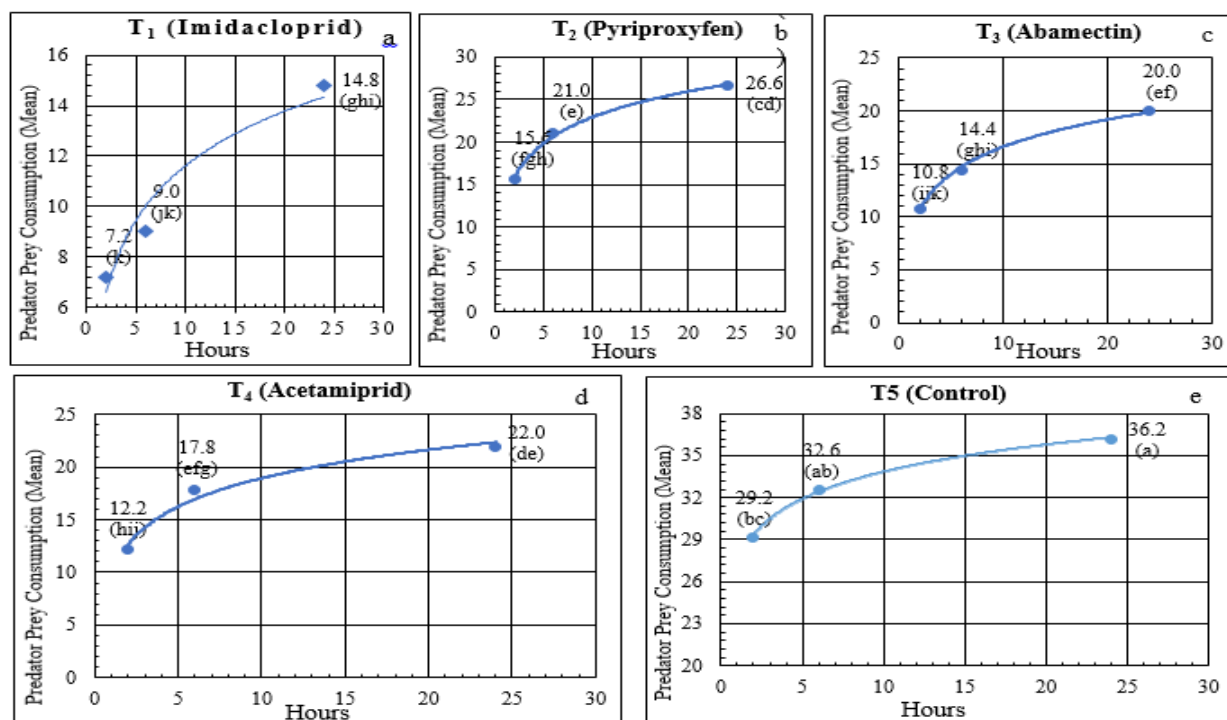
(Annexure C)

Figure 3 Mean consumption of *Coccinella septempunctata* females after the application of different insecticides at various intervals.



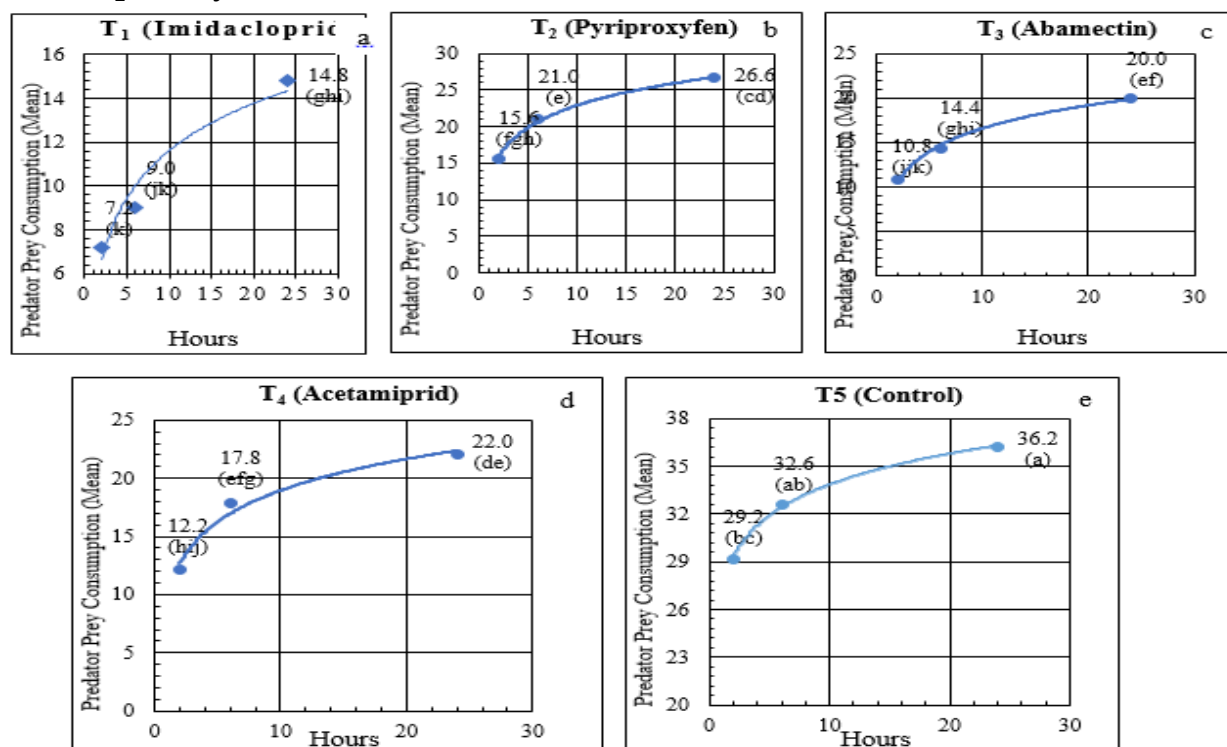
(Annexure D)

Figure 4 Mortality percentage of aphids in treated plants during predatory efficiency of *Coccinella septempunctata* females at various intervals



(Annexure E)

Figure 5 Foraging behavior of predator *Coccinella septempunctata* males on aphids in treated plant system at different intervals



(Annexure F)

Figure 6 Foraging behavior of predator *Coccinella septempunctata* females on aphids in treated plant system at different intervals

Supplementary table

Factor	F-value	Degrees of Freedom	p-value
Consumption (Treatment)	35.6	(4, 25)	<0.001
Consumption (Time)	16.3	(2, 25)	<0.001
Consumption (Interaction)	6.8	(8, 25)	<0.001
Aphid Mortality (Treatment)	49.2	(4, 25)	<0.001
Aphid Mortality (Time)	20.4	(2, 25)	<0.001
Aphid Mortality (Interaction)	7.3	(8, 25)	<0.001
Foraging Behavior (Treatment)	42.1	(4, 25)	<0.001
Foraging Behavior (Interaction)	9.7	(8, 25)	<0.001