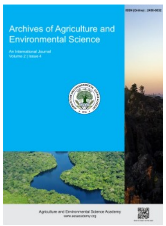
Archives of Agriculture and Environmental Science 10(1): 126-131 (2025)
https://doi.org/10.26832/24566632.2025.1001018

e-ISSN: 2456-6632

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: journals.aesacademy.org/index.php/aaes



ORIGINAL RESEARCH ARTICLE

CrossMark

In vitro comparative toxicity of chemical insecticides and botanicals against ladybird beetle (*Micraspis discolor* F.) in Nepal

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ARTICLE HISTORY

Received: 10 December 2024
Revised received: 27 February 2025
Accepted: 09 March 2025

Keywords

Botanical
Insecticides
Micraspis discolor
Mortality
Natural enemy

ABSTRACT

Micraspis discolor F. is the most abundant ladybird beetle in rice crop ecosystems and an effective natural predator of several economically significant agricultural pests. However, the widespread use of non-selective insecticides has disrupted natural enemy populations, highlighting the need for safer alternatives to integrate into pest management strategies. Limited research exists on the toxicity of commercial and bio-rational insecticides on beneficial insects. Therefore, this study aimed to evaluate the toxicity of various insecticides and botanicals in a laboratory setting to determine which pesticide is safest for *M. discolor* and to assess its mortality. The experiment followed a completely randomized design with five treatments: Azadirachtin 0.03% EC, garlic extract, Dimethoate 30% EC, Chlorpyrifos 50% EC + Cypermethrin 5% EC, and a control, each replicated five times. Mortality rates were recorded at 24, 48, and 72 hours' post-exposure. The results showed that *M. discolor* was significantly affected by Chlorpyrifos 50% EC + Cypermethrin 5% EC, which caused the highest mortality (86%) after 72 hours, followed by Dimethoate (74%). Among the tested substances, garlic extract was the safest, with a mortality rate of 54%, although still higher than the control, which exhibited no mortality. These findings highlight the potential of botanical extracts as safer alternatives for pest management, aiding in the conservation of beneficial insect populations. Furthermore, in order to promote sustainable agriculture, future research should concentrate on developing safer substitutes that reduce damage to pollinators, parasitoids, and natural predators while preserving efficient pest control.

Citation of this article: Khadka, P., & Karki, N. (2025). In vitro comparative toxicity of chemical insecticides and botanicals against ladybird beetle (*Micraspis discolor* F.) in Nepal. *Archives of Agriculture and Environmental Science*, *10*(1), 126-131, https://dx.doi.org/10.26832/24566632.2025.1001018

INTRODUCTION

One of the major global challenges today is ensuring high agricultural yields of superior quality while simultaneously maintaining sustainability in production. Achieving this balance necessitates a reevaluation of the role insects play in agricultural ecosystems. As key drivers of ecosystem functions, insects contribute significantly to agro-ecology—the practice of managing agricultural systems in an environmentally sound and sustainable manner by promoting ecosystem services (ES) delivered by beneficial organisms (Paywell *et al.*, 2015). Insects are crucial due to their vast diversity, ecological significance, and impact on agriculture, human health, and natural resources (Adetundan & Olusola, 2013). Within agriculture, insects can be broadly categorized as beneficial or non-beneficial. Beneficial insects include pollinators as well as predators and parasitoids that regulate pest populations. Among these, coccinellids have garnered considerable attention due to their capacity to utilize alternative food sources during non-cropping periods when pest populations are low (Shanker *et al.*, 2013). The term "ladybird" has been used for over 600 years in England to refer to the European beetle *Coccinella septempunctata* L., and as entomological knowledge expanded, it became a common name for all members of the beetle family Coccinellidae (Frank & Mizell, 2008). A ladybird beetle is also known as a ladybug in North America or ladybird in other parts of the world. Among insect predators, the ladybugs (Coleoptera: Coccinellidae) include major predators of harmful arthropods in agriculture (Joshi *et al*., 2012). *M. discolor* exhibits a diverse host range and distinct prey preferences. Pervez (2001) observed the prey preference of *Micraspis dicolor* F. among nine aphid preys and found *Lipapis erysimi* (Mustard aphid) to be preferred by ladybird beetle over several other aphids. It was also observed in okra, cow pea, mustard cabbage and rice field (Rajan *et al*., 2019). It can be effectively used as a bio-control agent of the tea aphid and can be used in integrated pest management programs successfully (Roy & Rahman, 2014).

The application of insecticides is a widely adopted method by farmers globally to manage insect pests. However, the excessive and frequent use of pesticides has led to several negative consequences, including biodiversity loss, secondary pest outbreaks, insecticide resistance in pests, residual toxicity, and disruption of natural enemy populations (Driesche & Bellows, 1996). The indiscriminate use of broad-spectrum pesticides in agriculture significantly impacts biological control agents, which play a crucial role in pest management (Carmo *et al*., 2010). A key consequence of frequent pesticide application is the proliferation of secondary pest species that are not directly targeted by insecticides but thrive due to reduced competition or diminished predation pressure (Dutcher, 2007). In contemporary farming systems, the population of natural enemies has declined due to post-harvest plant removal and pesticide exposure. Beneficial insects can be killed by synthetic pesticides, with direct killing being the primary effect. Because plant-feeding insects may have detoxifying mechanisms provided by plants, predators and parasitoids are typically more vulnerable to pesticides. Reducing these adverse effects on natural enemies requires the careful selection of pesticides that are as compatible as possible with biological control agents (El-Wakeil *et al*., 2017). Research indicates that both synthetic and botanical pesticides negatively affect natural enemies and pollinators in agricultural ecosystems. These impacts include reduced survival rates across different life stages, decreased reproductive potential, alterations in host suitability for parasitism or predation, lower parasitoid emergence from treated host eggs, and direct mortality (Ndakidemi *et al*., 2016). Similarly, natural plant products have some insecticidal properties as well. The insecticidal properties of natural plant products have been known since ancient times. It is estimated that over 2,000 plant species possess biological activity against insects, and principal chemicals that impart such activity include alkaloids, terpenoids, acetogenins and flavonoids (Sharma *et al*., 2012).

The careful application of pesticides is a crucial strategy for managing pests while minimizing harm to beneficial biological components. Choosing an appropriate pesticide requires a thorough evaluation of its effectiveness in controlling pests and its selectivity toward beneficial organisms, such as predators and parasitoids, which play a vital role in regulating pest populations (Fernandes *et al*., 2010). The use of selective insecticides can help conserve natural enemies, thereby enhancing the efficiency of integrated pest management (IPM) programs (Khan *et al*., 2015). Pesticides have different modes of action, influencing not only their direct lethality to arthropod pests but also their indirect effects on populations of beneficial insects (Ware & DM, 2005). The type of pesticide used significantly impacts natural enemies; for example, broad-spectrum nerve toxin pesticides—such as older compounds from the organophosphate, carbamate, and pyrethroid classes—tend to be more hazardous to beneficial organisms than non-nerve toxin alternatives (Cloyd, 2006). A study by Mollah *et al*. (2013) found that Esfenvalerate % EC at 1.0 ml/L caused the highest mortality in ladybird beetles, whereas Emamectin benzoate 5 SG at 1.0 g/L exhibited the lowest toxicity among the tested insecticides. Similarly, conventional insecticides like Dursban led to 50% mortality in ladybird beetles within a short period (Al-Doghairi *et al*., 2004). The successful integration of pesticides with natural enemies depends on several factors, including the pesticide class, the species of the natural enemy, the formulation of the pesticide, and the concentration to which these beneficial organisms are exposed. While pesticides and natural enemies have often been considered conflicting elements in pest control, there is significant potential to integrate both within sustainable pest management strategies to prevent secondary pest outbreaks caused by excessive reliance on chemical control (Ruberson *et al*., 1998). Given the increasing use of pesticides in agro-ecosystems, selecting environmentally safer alternatives is essential. However, the toxicity of commercial and bio-rational pesticides on beneficial insects remains insufficiently studied. Therefore, this study aims to evaluate the toxicity of various pesticides under laboratory conditions to identify the safest option for *Micraspis discolor* (F.) and to assess its mortality rate.

MATERIALS AND METHODS

Experimental details

The experiment was conducted in the laboratory of the Department of Entomology at the Institute of Agriculture and Animal Sciences, Lamjung Campus, from January 10 to March 15, 2024. It followed a completely randomized design with five treatments, each replicated five times. The treatments included two chemical insecticides, two botanical pesticides and a control for comparison. The details of the treatments, along with their formulations, are represented in Table 1.

Table 1. Treatments with their formulations used in the study.

Treatment	Pesticide	Formulations
T1	Control	-
T2	Nimbecide (Azadiractin 0.03%)	0.03% EC
T3	Garlic Extract	-
T4	Dimethoate (30% EC)	30% EC
T5	Chlorpyrifos 50% + Cypermethrin 5% EC	50% +5 % EC

Table 2. Preparation of insecticide for experiment.

Treatment No.	Treatment	Concentration
T1	Control	Normal water was used for the spray
T2	Nimbecide (Azadiractin 0.03%)	3 ml/Liter of water
T3	Garlic Extract	50ml/2.5 liter of water
T4	Dimethoate (30% EC)	1 ml/liter of water
T5	Chlorpyrifos 50% + Cypermethrin 5% EC	2 ml/liter of water

Beetle collection and rearing

Adult ladybird beetles (*Micraspis discolor* F.) were collected from an untreated mustard field in Sundarbazar-7, Lamjung. They were placed in perforated plastic jars covered with muslin cloth and fed an ad libitum diet of mustard aphids (*Lipaphis erysimi*). Before the experiment, careful observations were conducted to ensure that the beetles were free from physical injuries. Following the topical application of insecticides, each ladybird beetle was provided with 30 mustard aphids per individual, amounting to 300 aphids per petri dish. Additionally, a cotton pad soaked in honey was supplied as an alternative food source every 24 hours. Fresh batches of aphids were introduced at the end of each 24-hour period.

Preparation and application of insecticide

The experiment utilized commercially available pesticides, including Nimbecide (Azadirachtin 0.03%), Garlic extract, Dimethoate (30% EC), and a combination of Chlorpyrifos (50%) and Cypermethrin (5% EC). The garlic extract was prepared in the laboratory by grinding 50 grams of garlic using a mortar and pestle, soaking it in one liter of water for 24 hours, and then filtering it for use. Pesticides were applied following the recommended concentrations specified on their labels. Petri dishes were initially set up with filter papers before placing ladybird beetles inside them. To simulate the topical application of insecticides, plastic syringes were used. Each treatment involved applying 2 ml of pesticide solution, diluted according to the label recommendations. Table 2 outlines the insecticide preparation process for the experiment.

Observation, data collection, and statistical data analysis

The data were collected after 12hours, 24 hours, 48 hours, and 72 hours of exposure for the number of dead ladybird beetles. For confirming the death, visual observation was done and the abdomen was observed if any doubt to ensure there is no respiration. The data collected were entered using MS-Excel 2016 and analyzed by Fisher test in one factorial ANOVA using R statistics 3.13 software package. The mean separation was performed by Duncan's Multiple Range Test (DMRT) at 0.05 confidence level.

The mortality is estimated by counting the total number of dead and live insects:

Mortality % = (Number of dead individuals / Total individuals) × 100

The parallel check i.e. control has zero mortality therefore no mortality correction was used.

RESULTS AND DISCUSSION

Mortality observed after 12 hours

After 12 hours of experimental setup, the mortality of *M. discolor* resulted by various pesticides followed the order: Chlorpyrifos 50% + Cypermethrin 5% > Dimethoate > Nimbecide > Garlic Extract > Control. It was observed that no ladybird beetles were found dead i.e. 0% of mortality in the control. Among the pesticides, Garlic extract has the least mortality which was statistically at par with mortality due to Nembecide with mortality of 16%. The highest mortality was found in Chlorpyrifos 50EC+ Cypermethrin 5% EC which was followed by Dimethoate with 28% mortality which was significantly different.

Mortality observed after 24 hours

After 24 hours, it was observed that Chlorpyrifos 50% EC + Cypermethrin 5% EC resulted to highest mortality i.e. 62% which was followed by Dimethoate among which the significant differences were observed. The lowest mortality was observed in Garlic Extract i.e. 26% and the mortality due to Nimbecide was 28% which was statistically indifferent with Garlic Extract. The mortality at 48 hours followed the following order: Chloropyrifos 50% EC + Cypermethrin 5% EC > Dimethoate 30% EC > Nimbecide > Garlic Extract > Control.

Mortality observed at 48 hours

After 48 hours, it was observed that Chlorpyrifos 50% +Cypermethrin 5% EC resulted in highest mortality i.e. 78%. The control had 0% mortality. Among the pesticides, Nimbecide had the least mortality with 38% which was statistically at par with Garlic extract which resulted in the mortality of 46%. The mortality due to Dimethoate (30% EC) was 68%. The mortality due to various treatment at 48 hours of observation was found as: Chlorpyrifos 50 % + Cypermethrin 5% EC > Dimethoate (30 % EC) > Nimbecide > Garlic extract.

Mortality after 72 hours

After 72 hours, the mortality due to pesticides over the beneficial ladybird beetles (*M. discolor*) were found to be significantly different at 0.05% level of confidence. It was found that Chlorpyrifos 50% EC + Cypermethrin 5% EC resulted in the highest mortality with mean mortality of 86% followed by Dimethoate 30 EC with 74% mortality which were significantly different. Garlic extract resulted in least mortality with mortality of 54% which followed Nimbecide with mortality percentage of 64% and were significantly different. The effect over mortality due to Garlic extract and Nembicide was found to be signifi-

Table 3. Mortality of ladybird beetle (*M. discolor*) under different observations.

Treatment	Mortality percentage			
	12 hours	**24 hours**	**48 hours**	**72 hours**
Control	0d	0d	0e	0e
Nimbecide	16^{c}	26^{c}	46^{c}	62^{c}
Garlic Extract	14^{c}	26^{c}	36d	46^{d}
Dimethoate (30% EC)	28^{b}	52^{b}	68^{b}	74^{b}
Chlorpyrifos 50% EC+ Cypermethrin 5% EC	44^{a}	62^{a}	78^{a}	86^{a}
LSD	9.69	9.32	8.34	8.54
CV%	22.13	21.29	13.86	12.09
F test	***	***	***	***

Note: *, ** and *** denote significance at p=0.050, P=0.01, P<0.001 respectively, CV= coefficient of Variation, LSD =Least Significant Difference, Treatment means within a column followed by the different letter were significantly different at p=0.05 level by Fischer LSD.

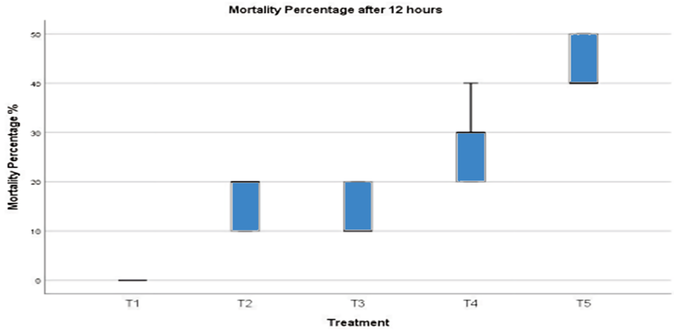

Figure 1. *Mortality percentage of ladybird beetle (M. discolor) after 12 hours.*

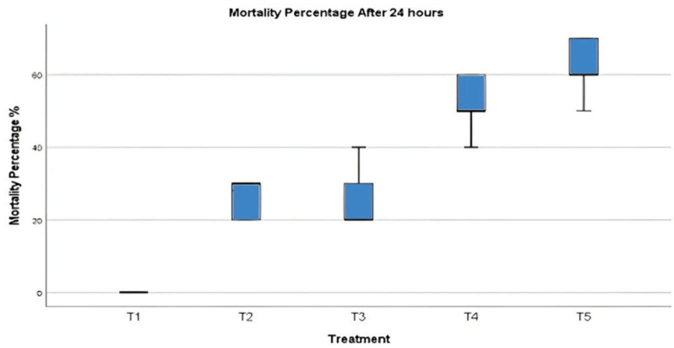

Figure 2. *Mortality percentage of ladybird beetle (M. discolor) after 24 hours.*

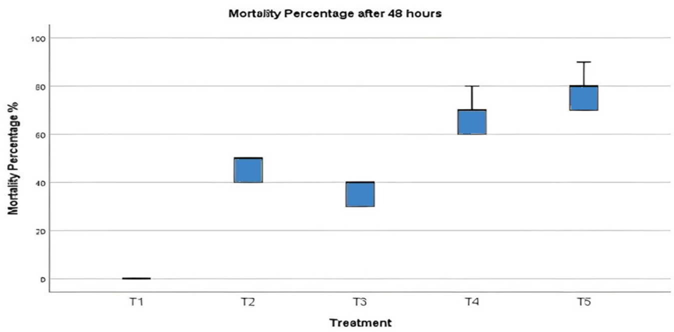

Figure 3. *Mortality percentage of ladybird beetle (M. discolor) after 48 hours.*

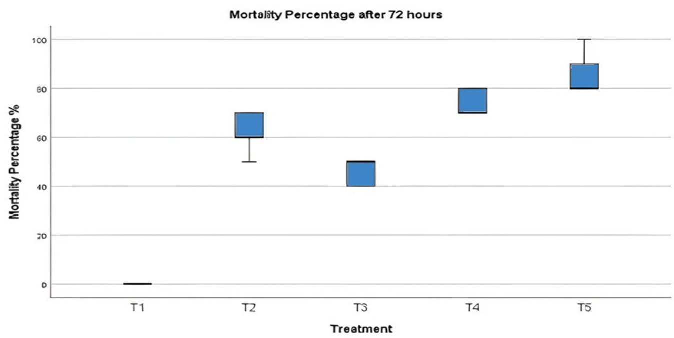

Figure 4. *Mortality percentage of ladybird beetle (M. discolor) after 72 hours.*

cantly different. It was also observed that the control had no mortality at 72 hours of experiment which ensures the mortality due to pesticides over the natural mortality. The mortality percentage in the beneficial ladybird beetles followed the order as follows: Chlorpyrifos 50% + Cypermethrin 5 % EC > Dimethoate 30 % EC > Nimbecide > Garlic Extract > Control. The toxicity data of various insecticides on the mortality of *M. discolor* revealed that the combination of Chlorpyrifos 50% EC + Cypermethrin 5% EC resulted in significantly higher mortality after 72 hours compared to other insecticides. These results are consistent with those of Mughal *et al.* (2017), who identified Chlorpyrifos as the most toxic insecticide to the adults of the seven-spotted beetle, *Coccinella septempunctata* L., and Khan *et al.* (2015), who found that Cypermethrin exhibited high toxicity, causing maximum mortality in the egg, larva, pupa, and adult stages of the zigzag ladybird beetle, *Menochilus sexmaculatus*. Coats *et al.* (1979), in their study on the toxicity of three synthetic pyrethroids to eight species of predatory coccinellids, concluded that Cypermethrin was the most toxic to ladybird beetles. Zhou *et al.* (2011) also observed that the combined effect of Cypermethrin and Chlorpyrifos was significantly more toxic than either pesticide used individually, which aligns with our findings that the combination of Chlorpyrifos 50% EC and Cypermethrin 5% EC resulted in significantly higher mortality. Mortality due to Dimethoate 30% EC was the second highest, differing significantly from the mortality caused by Chlorpyrifos 50% EC + Cypermethrin 5% EC. At each observation interval, Dimethoate 30% EC resulted in significantly higher mortality than Nimbecide. Shanmugapriya & Muralidharan, (2017) reported that Dimethoate 30% EC, commonly used against sucking pests, resulted in significantly higher mortality than Nimbecide, which proved to be safer. Nimbecide (Azadirachtin 0.03%) showed a significant impact on the mortality of adult ladybird beetles, similar to garlic extract. Also, Mochia *et al.* (2011) reported the non-harmful effects of EcoGold 999plus, neem oil, and garlic bulb extract on ladybird beetles and huntsman spiders, which are natural enemies of whiteflies and aphids. Both Nimbecide and garlic extract had a significant impact on mortality; after 72 hours, Nimbecide (Azadirachtin 0.03%) caused 62% mortality, while garlic extract resulted in 46%, both significantly different from the control. Medina *et al.* (2003) found that Azadirachtin was harmful to the lacewing *Chrysoperla carnea* (Stephens). Similarly, Swaminathan *et al.* (2010) observed that Neem Seed Kernel Extract (NSKE 10%) resulted in the highest mortality. Overall, our results suggest that garlic extract was the safest among all insecticides and botanicals, although it still caused significant mortality compared to the control, where no mortality occurred. These findings are consistent with those of Mamduh *et al.* (2017), who recommended garlic extract as a safer option for natural enemies involved in pest control. Garlic crude extract also caused mortality in *Coccinella carnea* and *Coccinella septempunctata*, with rates of 65% and 20%, respectively reported earlier by Nasseh *et al.* (1993). This indicates that garlic extract has some effect on natural enemies like ladybird beetles, which is in line with our findings of 46% mortality after 72 hours of exposure.

Conclusion

The experiment aimed to evaluate the in-vitro comparative toxicity of various chemical insecticides and botanicals against adult ladybird beetles, *M. discolor*, a valuable predator of pests such as brown plant hopper, tea aphids, bean aphids, and mustard aphids. After 72 hours of exposure, it was found that Chlorpyrifos 50% + Cypermethrin 5% caused the highest mortality, followed by Dimethoate 30% EC. The lowest mortality was observed with garlic extract, followed by Azadirachtin 0.03% EC, while the control group showed no mortality. It was concluded that none of the insecticides and botanicals tested, except the control, were completely safe for the adults of *M. discolor*. However, garlic extract was the safest among the pesticides evaluated, followed by Nimbecide. Both botanicals and synthetic pesticides caused mortality in the insects responsible for pest control. While the negative effects of synthetic pesticides are well-documented, botanicals are generally considered safer. Nevertheless, there is a need to develop safer pesticides for ladybird beetles, along with other natural predators, parasitoids, and pollinators, to enhance the effectiveness of integrated pest management. Additionally, future efforts should focus on creating safer alternatives that minimize harm to natural predators, parasitoids, and pollinators while maintaining effective pest management to support sustainable agriculture.

ACKNOWLEDGEMENTS

We would like to thank the staffs of the Institute of Agriculture and Animal Sciences, Lamjung Campus, for their relentless technical support throughout the strenuous research work, as well as for their assistance during times of need and difficulty.

DECLARATIONS

Authors contributions statement

Conceptualization: P.K. and N.K.; Methodology: N.K.; Software and validation: NK; Formal analysis and investigation: P.K. and N.K.; Data curation: N.K.; Writing—original draft preparation: P.K.; Writing—review and editing: P.K., and N.K.; Visualization: N.K.; Supervision: P.K. and N.K. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethics approval: This study did not involve any animal or human participant and thus ethical approval was not applicable.

Consent for publication: All co-authors consented to publish this paper in AAES.

Data availability: The data supporting this study's findings are available on request from the corresponding author.

Supplementary data: No supplementary data is available for the paper.

Funding statement: No external funding is available for this study.

Additional information: No additional information is available for this paper.

Publisher's Note: Agro Environ Media (AESA) remains neutral with regard to jurisdictional claims in published maps, figures and institutional affiliations.

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