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COMPARATIVE TOXICITY AND RISK ASSESSMENT OF SOME PESTICIDES ON THE PARASITOID, *Habrobracon hebetor* (Say)

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ABSTRACT

Habrobracon hebetor **(Say) is a widely used gregarious ectoparasitoid in biological control programs targeting lepidopteran pests. However, the extensive use of chemical pesticides poses potential risks to such beneficial organisms. This study assessed the lethal and sublethal effects of four commonly used pesticides-imidacloprid, acephate, propiconazole, and fenoxaprop-p-ethyl on adult *H. hebetor* through concentration-mortality assays and risk quotient (RQ) analysis. Among the tested compounds, acephate was the most toxic, with LC_5, LC_{30}, and LC_{50} values of 0.01, 0.15, and 0.52 mg L^{-1}, respectively, and an RQ value of 1442.31, indicating a high ecological risk. In contrast, imidacloprid exhibited LC_5, LC_{30}, and LC_{50} values of 0.27, 8.27, and 40.75 mg L^{-1}, respectively, with an RQ of 0.61, suggesting minimal risk. The fungicide propiconazole (LC_{50}: 23.79 mg L^{-1}; RQ: 5.25) and the herbicide fenoxaprop-p-ethyl (LC_{50}: 995.41 mg L^{-1}; RQ: 0.06) also showed low toxicity, with sublethal concentrations well above the field-relevant levels. These results underscore the importance of incorporating both lethal and sublethal toxicity endpoints when evaluating pesticide compatibility with natural enemies. Future studies should explore long-term sublethal impacts on reproductive, behavioural, and molecular traits to support sustainable IPM strategies.**

Keywords: Bioassay; biological control; hazard quotient; natural enemy; risk assessment

INTRODUCTION

The application of chemical pesticides continues to be a predominant strategy for managing agricultural pests due to their rapid action, cost-effectiveness, and high efficacy (Umetsu and Shirai, 2020). However, indiscriminate and excessive use of pesticides has contributed to the development of resistance in target pest populations, often leading to secondary pest outbreaks (Stanley and Preetha, 2016). In addition, chemical pesticides frequently exert detrimental effects on non-target beneficial organisms, particularly natural enemies, which are closely associated with pest populations (Torres and Bueno, 2018; Ricupero *et al*., 2020). Natural enemies can be more sensitive to insecticides than the pests they help control (Preetha *et al*., 2010).

The conservation of natural enemies is recognized as a cornerstone of biological pest control and forms one of the key components of integrated pest management (IPM) strategies (Mills, 2014). Insecticides are a vital tool in pest control, although some fungicides too possess insecticidal properties, potentially impacting beneficial arthropods (Amarasekare and Shearer, 2013a,b). Hence, it is essential to generate comprehensive data on both the lethal and sublethal effects of reduced-risk pesticides on natural enemies to ensure their compatibility with biological control agents (Jones *et al*., 2009).

Pesticide selectivity towards natural enemies can be evaluated in both laboratory and field conditions. However, laboratory assessments are more commonly employed due to the complexity and variability of biotic and abiotic factors in field environments (Beers *et al*., 2015). Integrating chemical and biological control strategies is essential for sustainable pest management. This requires a thorough understanding of interactions between insecticides and natural enemies to facilitate their effective coexistence within IPM frameworks (Wright and Verkerk, 1995; Greathead, 1995).

Habrobracon hebetor (Say) (Hymenoptera: Braconidae) is a gregarious larval ectoparasitoid that parasitizes a wide range of Lepidopteran hosts (Nath *et al*., 2023). Its high fecundity, short generation time, and broad host range makes it an effective biological control agent in both field and storage systems (Ghimire and Phillips, 2014; Noosidum *et al*., 2020; Ou *et al*., 2023). For laboratory rearing purposes, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) is widely used as a standard host due to its compatibility and ease of handling (Alam *et al*., 2014). *H. hebetor* is a prominent larval ectoparasitoid of rice leaf folder, *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), a major insect pest in rice cultivation that contributes substantially to yield losses (Gurr *et al*., 2012; Huang *et al*., 2012). To manage such pests, rice agroecosystems are frequently subjected to chemical interventions, including insecticides, fungicides, and herbicides. However, extensive and indiscriminate use of these agrochemicals can lead to unintended ecological consequences, notably cross-toxicity, which negatively impacts non-target beneficial arthropods such as parasitoids and predators (Lu *et al*., 2012; Wyckhuys *et al*., 2013). Although the deleterious effects of insecticides on natural enemies are relatively well-documented, the potential sublethal and chronic impacts of other pesticide categories, particularly fungicides and herbicides, remain insufficiently studied. Comprehensive assessment of these lesser-studied chemical groups is imperative for understanding their potential to disrupt the biological performance of key natural enemies and to refine integrated pest management (IPM) strategies for sustainable rice production. In this context, the present study was aimed to evaluate the toxicity and risk associated with four commonly used pesticides namely imidacloprid, acephate, propiconazole, and fenoxaprop-p-ethyl on *H. hebetor*. The study focused on assessing both lethal and sublethal effects to support informed decisions on pesticide use within IPM systems.

MATERIALS AND METHODS

Source and rearing of factitious host

Initial cultures of *C. cephalonica* were obtained from the Biocontrol Laboratory, Crop Protection Division, ICAR-Central Rice Research Institute (CRRI), Cuttack (India). Rearing was conducted following the procedure of Gowda *et al*. (2021a). For mass production, 0.25 cc eggs (~ 5000 eggs) were introduced into a rearing medium composed of 2.5 kg sterilized, pesticide-free broken maize grains, 50 g roasted groundnut powder, 2 g yeast, 5 g multivitamin powder, 0.2 g streptomycin sulphate, and 10 mL 0.1% formalin. Upon adult emergence, moths were collected using a suction system powered by a 120 W motor, consisting of a 50 L outer plastic container and a 10 L inner oviposition chamber. A 15-mesh wire screen was fitted at the base of the chamber to facilitate egg collection. To maintain the moths, a cotton swab soaked in a 50% honey solution was affixed inside the oviposition chamber. Eggs deposited at the chamber's base were collected and sieved 3-4 times to remove debris before use in further experiments.

Rearing of parasitoid

H. hebetor was reared following the protocol outlined by Pradhan *et al*. (2025). A mating pair (one male and one female) was placed in a 1000 mL plastic container and allowed to mate for 24 h (Gowda *et al*., 2023). For parasitization, five late-instar larvae (4th and 5th instars) of *C. cephalonica* (Hagstrum and Smittle, 1977) were positioned over the container's opening in a sandwich method (Ghimire and Phillips, 2014). A 50% honey solution, provided on a cotton swab, served as adult food source (Abedi *et al*., 2014). After 48 h exposure, parasitized larvae were transferred to an insect growth chamber maintained at 25±1℃, 70±5% relative humidity, and a 14:10 h light: dark photoperiod to ensure successful development of the parasitoids.

Pesticides and reagents

Four widely used pesticides representing different chemical classes were selected to evaluate their toxicity to *H. hebetor*: imidacloprid and acephate (insecticides), propiconazole (fungicide), and fenoxaprop-p-ethyl (herbicide). The test pesticides, procured from Sigma Aldrich, St. Louis, USA, were chosen based on their prevalent usage in contemporary pest management practices. Technical grade (98%) pesticides were used in bioassays to ensure precise dosing and accurate toxicity assessment of active ingredient, avoiding variability from inert formulation components and enabling consistent, reproducible dose-response results. Stock solutions were prepared by dissolving each active ingredient in acetone (analytical reagent grade).

Toxicity assay

The toxicity of the selected pesticides to *H. hebetor* was evaluated through a concentration-mortality bioassay, employing the dry film residue technique as standardized by Ray *et al*. (2023). Prior to the definitive tests, preliminary trials were conducted to identify the appropriate concentration ranges for each compound. The concentration range causing 10 to 90% adult mortality was established through preliminary bioassays. Based on preliminary trials, the desired test concentrations for each pesticide were prepared by diluting the respective stock solutions as follows: imidacloprid (10, 50, 100, 150, and 200 mg L^{-1}), acephate (0.1, 0.5, 1.0, 1.5, and 2.0 mg L^{-1}), propiconazole (10, 20, 30, 50, and 100 mg L^{-1}), and fenoxaprop-p-ethyl (800, 900, 1000, 1100, and 1200 mg L^{-1}). To ensure uniform deposition, 1 mL of each pesticide solution was applied to the inner surface of glass tubes (Borosil®; dimensions: 19.0 cm height × 3.6 cm diameter). The tubes were gently rotated to achieve even coverage and left undisturbed at room temperature for 1 h to allow complete evaporation of solvent, resulting in a thin and consistent pesticide film. Following the drying period, sixty newly emerged adult *H. hebetor* (< 24 h old) were introduced into each treated tube. A thin streak of honey was provided as a nutritional source, and the tube openings were sealed with muslin cloth to allow ventilation while preventing escape. The tubes were then incubated under controlled laboratory conditions in an insect growth chamber (JSPC-420C, JS Research Inc., Gongju, Republic of Korea) maintained at 25±1°C, 70±5% relative humidity, and a photoperiod of 14 h light and 10 h dark. Each concentration treatment was replicated three times, using independent tubes. After a 24 h exposure, the wasps were carefully transferred to pesticide-free tubes containing fresh honey streaks to prevent any further exposure. Mortality was assessed by gently probing each individual with a fine brush, and those failing to exhibit movement were recorded as dead. The percentage mortality was then calculated relative to the initial number of insects introduced in each replicate.

Risk assessment

The ecological risk posed by pesticides to *H. hebetor* was evaluated using the risk quotient (RQ) method as described by Preetha *et al*. (2009):

$$\text{Risk quotient} = \frac{\text{Recommended field rate (g a.i./h)}}{\text{LC50 of beneficial insect (m a.i./l)}}$$

The RQ values provide a quantitative measure of potential pesticide risk to non-target organisms, where $RQ < 50$ is generally considered harmless, $50 \leq RQ \leq 250$ moderately toxic, and $RQ > 250$

harmful. The recommended field application rate of imidacloprid, acephate, propiconazole, and fenoxaprop-p-ethyl are 25, 750, 125 and 56.25 g a.i. h^{-1}.

Statistical analysis

The mortality data were subjected to probit analysis using PoloPlus version 2.0 (LeOra Software Inc., Berkeley, CA, USA) to estimate the lethal concentrations (LC_5, LC_{30}, LC_{50}, and LC_{90}) and their respective 95% fiducial limits (Qu *et al*., 2015; Ullah *et al*., 2019).

RESULTS AND DISCUSSION

The concentration–mortality bioassay revealed significant variation in the toxicity profiles of test pesticides against adult *H. hebetor*, encompassing insecticides, a fungicide, and a herbicide (Fig. 1, Table 1). The median lethal concentrations (LC_{50}) for these compounds were found to be 40.75, 0.52, 23.79, and 995.41 mg L^{-1}, respectively. These findings indicate variable toxicity levels among the tested pesticides, with acephate showing the highest toxicity and fenoxaprop-p-ethyl the lowest. The LC_{50} value for imidacloprid observed in this study closely aligned with the results of Sarmadi *et al*. (2010), who reported an LC_{50} of 42.13 mg L^{-1} for *H. hebetor*. In contrast, Shankarganesh *et al*. (2017) documented a substantially lower LC_{50} (9.5 mg L^{-1}) for imidacloprid against another braconid parasitoid, *Bracon brevicornis* (Wesmael) (Hymenoptera: Braconidae), highlighting potential interspecific variation in susceptibility.

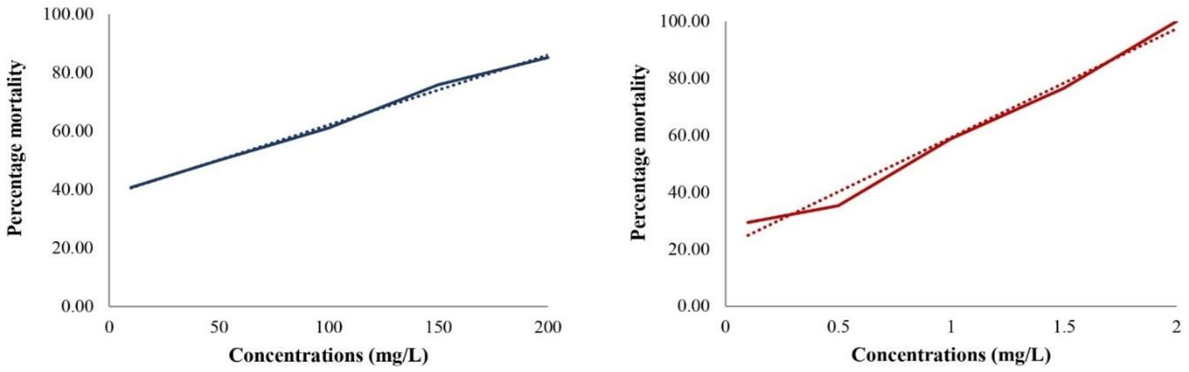
Table 1: Concentration-mortality assay of different pesticides on *Habrobracon hebetor* adults

Pesticides	n	Slope ± SE	LC_5 [mg L^{-1}] (95% FL)	LC_{30} [mg L^{-1}] (95% FL)	LC_{50} [mg L^{-1}] (95% FL)	LC_{90} [mg L^{-1}] (95% FL)	χ^2 (df)
Imidacloprid	60	0.766 ± 0.238	0.27 (0.09-0.80)	8.27 (2.82-24.24)	40.75 (13.91-119.38)	2006.34 (684.88-5877.52)	0.831 (3)
Acephate	60	0.980 ± 0.188	0.01 (0.004-0.024)	0.15 (0.06-0.34)	0.52 (0.22-1.20)	11.08 (4.74-25.90)	0.146 (2)
Propiconazole	60	1.524 ± 0.123	1.98 (1.14-3.44)	10.76 (6.19-18.72)	23.79 (13.68-41.36)	165.14 (94.97-287.16)	0.998 (3)
Fenoxaprop-p-ethyl	60	6.666 ± 0.027	563.9 (498.2-638.2)	830.5 (733.8-939.8)	995.4 (879.5-1126.5)	1549.9 (1369.5-1754.1)	0.999 (3)

SE: Standard error; LC: Lethal concentration; FL: Fiducial limits; df: Degree of freedom

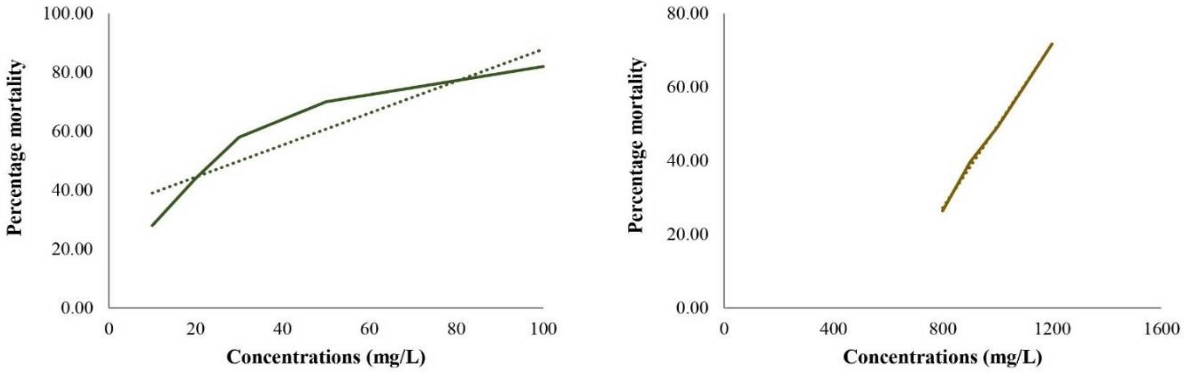
Greater susceptibility to imidacloprid has also been documented in other parasitoids. For instance, Ray *et al*. (2022) reported LC_{50} values as low as 2 μg L^{-1} for imidacloprid in *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae), consistent with the findings of Preetha *et al*. (2009), who recorded an LC_{50} of 0.0027 mg L^{-1} for the same species. Comparable high sensitivity to imidacloprid has been reported in *Trichogramma brasiliensis* (Hymenoptera: Trichogrammatidae) (Shankarganesh *et al*., 2013). Similarly, acephate exhibited significant toxicity to *H. hebetor* in this study, corroborated by the observations of Ramos *et al*. (2018), who reported 76-78% mortality in *Copidosoma truncatellum* (Hymenoptera: Encyrtidae) following acephate exposure.

Concerning fungicides, propiconazole was found to be less toxic to *H. hebetor* compared to the insecticides tested. This observation is supported by Khan *et al*. (2015), who reported limited toxicity of tebuconazole to *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). Similarly, Tai *et al*. (2022) found that both propiconazole and difenoconazole had minimal adverse effects on *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae), while Fontes *et al*. (2018) classified azoxystrobin as non-toxic to *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae). Nonetheless, Felton and Dahlman (1984) documented significant toxicity of *Microplitis croceipes* (Hymenoptera: Braconidae), suggesting that toxicity varies greatly depending on the active ingredient and parasitoid species.



Imidacloprid

Acephate



Propiconazole

Fenoxaprop-p-ethyl

Fig. 1: The percentage mortality of *Habrobracon hebetor* after adults were exposed to different concentrations of the four pesticides

Among the tested pesticides, fenoxaprop-p-ethyl exhibited the lowest toxicity to *H. hebetor*, with LC_{50} of 995.41 mg L^{-1} (Table 2). This contrasts with findings by Ray *et al*. (2022), who reported a much lower LC_{50} of 7.81 mg L^{-1} for the herbicide bispyribac sodium against *T. chilonis*, underscoring species-specific differences in herbicide sensitivity.

While assessing lethal concentrations provides valuable insights into pesticide risks, the sublethal effects that occur following pesticide degradation or environmental dilution also play a critical role in shaping ecological outcomes (Cutler, 2013). Sublethal exposures may elicit both detrimental and stimulatory effects on insect physiology and behaviour (Guedes *et al*., 2016; Gowda *et al*., 2021b). In present study, the LC_{30} and LC_5 values were observed to be 8.27, 0.15, 10.76, and 830.45 mg L^{-1}, and 0.27, 0.01, 1.98, and 563.89 mg L^{-1}, respectively, for imidacloprid, acephate, propiconazole, and fenoxaprop-p-ethyl. These values are in agreement with the findings of Ray *et al*. (2022), who reported an LC_5 of 0.07 µg L^{-1} for imidacloprid in another parasitoid, *T. chilonis*. Additionally, Faal-Mohammadali *et al*. (2014) demonstrated LC_{25} values of 0.04 mg L^{-1} for chlorpyrifos and 0.38 mg L^{-1}

Table 2: Risk assessments of commonly used pesticides for *Habrobracon hebetor*

Active ingredient	LC_{50} (mg L^{-1})	RQ value	Risk
Imidacloprid	40.75	0.61	Harmless
Acephate	0.52	1442.31	Moderately toxic
Propiconazole	23.79	5.25	Harmless
Fenoxaprop-p-ethyl	995.41	0.06	Harmless

RQ: Risk quotient

for fenpropathrin against *H. hebetor*, supporting the patterns observed in this study.

To further evaluate the ecological safety of test pesticides on *H. hebetor*, Risk Quotient (RQ) values were calculated. Among the tested compounds, acephate exhibited a notably high RQ value of 1442.31, classifying it as moderately toxic to *H. hebetor* and indicating a substantial ecological risk when applied in environments supporting this beneficial parasitoid. In contrast, imidacloprid yielded an RQ value of 0.61, categorizing it as harmless, despite its higher LC_{50} compared to acephate. Among the non-insecticidal pesticides, propiconazole had an RQ value of 5.25, and fenoxaprop-p-ethyl exhibited the lowest RQ value at 0.06. Both values fall well below the harm threshold, indicating that these compounds pose negligible risk to *H. hebetor* under expected field exposure conditions. These findings highlight the importance of using selective pesticides in integrated pest management (IPM) to safeguard beneficial parasitoids. Future research should explore the sublethal effects on key traits such as behaviour, reproduction, and population dynamics in multiple generations. Molecular and biochemical studies are also recommended to elucidate the mechanisms underlying pesticide-induced stress responses and detoxification pathways in *H. hebetor*.

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