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Unintended effects of herbicide exposure on non-target beetle *Zygogramma bicolorata* Pallister, 1953, a bio-control agent of *Parthenium hysterophorus* weed

Neha Singh¹, Nikhil Maheshwari² and Ayesha Qamar^{1*}

Abstract

Background Herbicides are commonly used to safeguard crops against weed damage, but their effects on non-target organisms like *Zygogramma bicolorata*, a natural bio-control agent feeding on *Parthenium hysterophorus*, an invasive weed, have raised concerns. This study investigates the impact of pendimethalin, a widely used herbicide, on *Z. bicolorata*.

Results The results of the present study reveal adverse effects induced by pendimethalin on various life parameters of *Z. bicolorata*. Fecundity decreased by approximately 20.98%, while egg viability reduced by about 18.36%. Female lifespan decreased by approximately 11.18%, and male lifespan decreased by about 13.80%, with an associated increase in developmental time period. Oviposition days after pendimethalin treatment showed only a minor 9.18% decrease compared to the control group. Pendimethalin also negatively impacted the gut antioxidants of *Z. bicolorata*, resulting in oxidative stress that can harm gut health and disrupt biological processes. At the molecular level, it has also been observed the DNA damage in the midgut tissue of *Z. bicolorata*.

Conclusion This study underscores the unintended consequences of herbicide use on non-target organisms, emphasizing the potential threats to biodiversity. Evaluating chemical impacts on non-target organisms is crucial for ensuring responsible and sustainable chemical use in agriculture and other sectors.

Keywords Pendimethalin, *Zygogramma bicolorata*, *Parthenium hysterophorus*, Gut, Oxidative stress, DNA damage

Background

Pesticides are essential to agricultural development because they can reduce crop losses, improve food quality and production, and keep costs low (Aktar et al., 2009; Fenik et al., 2011; Strassemer et al., 2017). Despite being an essential component of contemporary farming,

pesticides can be hazardous to human health and the environment (Bagheri et al., 2018; Tudi et al., 2021). Herbicides control weeds and do lessen the load of weeds on the crop in agricultural fields. However, they also have an impact on non-target organisms which include beneficial insects as well as other type of insect groups which play a role in the ecosystem. Insects are diverse group of animals that have adapted to a wide range of environments and feeding habits. They play a vital role in ecosystems as pollinators (Khalifa et al., 2021), decomposers (Sharif & Qamar, 2022), and a food source for other animals.

The weed *Parthenium hysterophorus* originated in the southern USA and Central America and spread quickly due to its invasive nature. It is presently expanding its

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range throughout Asia and other non-native areas, where it is having negative effects on native crop production, human and animal health, and pasture productivity (Shabbir et al., 2019; Weyl et al., 2021). Its aggressive nature, prolific seed production, genetic diversity, resistance to stress, and allelopathic effects on competing plants make it a difficult weed to control (Bajwa et al., 2016; Putri, 2022). *Parthenium* has also been found to cause severe allergies and dermatitis in humans and livestock (Patel, 2011).

Effective biological control of weeds depends on the interaction of biotic and abiotic factors. For weed management, the use of herbicides that are safe for bio-control agents is recommended, as misuse of synthetic chemicals can have negative impacts on beneficial insects (Sánchez-Bayo, 2021). In addition to mortality and decreased fertility, these substances have been shown to shorten lifespans, change oviposition patterns, diminish egg viability, and delay development (Benamu et al., 2010). Our continued reliance on herbicides remains unabated due to the imperative need to sustain crop output, thus resulting in a significant issue of herbicide misuse (Mesnage & Zaller, 2021). Therefore, it is important to choose the right herbicides and dosages for the conservation ecology and populations of bio-control agents (Bruhl and Zaller, 2021). A possible global bio-control agent against the *Parthenium* weed is *Zygogramma bicolorata* Pallister, 1953 (Coleoptera: Chrysomelidae) (Gupta et al., 2004; Qadir & Qamar, 2019; Shree et al., 2021). *Z. bicolorata* engages in both polyandrous and polygamous mating with same-pair mating preferences. The male and female beetles have qualitative similarities in their cuticular hydrocarbon profiles but exhibit sex-dependent responses to synthetic straight-chain alkanes (Qadir et al., 2021). The non-target effects of the pesticide have been the subject of extensive research (Desneux et al., 2007; Garzón et al., 2015).

In 1977, Australia initiated the biological control of *Parthenium*, which involved the introduction of *Z. bicolorata*, a leaf-feeding beetle, from Mexico in 1980 (McFadyen & McClay, 1981). This strategy was later adopted in India in 1984, with the introduction of *Z. bicolorata* for the management of *Parthenium* (Dhileepan & Strathie, 2009). The Indian Institute of Horticultural Research (IIHR) in Bangalore was responsible for introducing the beetle, which rapidly became widespread within 3 years. These findings were reported by Jayanth and Nagarkatti in (1987) and subsequently confirmed by Jayanth and Visalakshy in (1996).

Numerous herbicides, including metribuzin, alachlor, atrazine, paraquat, pendimethalin (PND) and glyphosate, are used routinely in farming settings. Pendimethalin (30% EC), herbicide in the dinitroaniline family, is used

for the selective control of annual weeds and grasses in croplands. Highly stable and persistent, PND can still be found in the soil 300 days after application. PND takes anywhere from 24 to 98 days to completely degrade in soil, depending on the soil pH and the temperature (Strandberg & Scott-Fordsmand, 2004). The PND inhibits microtubule polymerization, which is essential for cell division in plants (Rose et al., 2016). *Harpalus rufipes*, a useful ground beetle, has its cellular immune response and haemocyte morphology disrupted by PND (Vommaro et al., 2021). The enzyme activities of *H. rufipes* were decreased when they were exposed to PND based herbicides (Giglio et al., 2019). Due to its stability and biomagnification in the ecosystem, PND has become a threat to both aquatic (Ahmad et al., 2016) and terrestrial (Belden et al., 2005; Chakravorty et al., 2015) animals (Vighi et al., 2017). It negatively affects terrestrial invertebrates like springtails, earthworms, pill bugs (Belden et al., 2005), and *Trichogramma ostrinae*, a parasitoid wasp (Tai et al., 2022) that serve as decomposers, detritivores, and parasitoids in the ecosystem.

We undertook this study to examine the influence of commercial formulation of pendimethalin on *Z. bicolorata* life parameters, antioxidant defence system in the gut, and genotoxicity, with the hypothesis that pendimethalin exposure has the potential to detrimentally affect *Z. bicolorata* life parameters, induce oxidative stress, and result in genotoxicity in midgut.

Materials and methods

Stock maintenance

The adults and larvae of *Z. bicolorata* were systematically gathered from diverse locations, including wastelands and roadsides in Aligarh, India. Subsequently, all collected individuals were carefully pooled together to create a single, representative population. This pooled population was then introduced into a controlled laboratory environment, where a self-sustaining culture was maintained. Under properly regulated conditions of temperature, humidity, and photoperiod, this laboratory culture flourished. Fresh apical *Parthenium* leaves, serving as both sustenance for larvae and adults and sites for egg deposition, were consistently supplied within glass jars. These glass jars were securely housed within a BOD incubator, where ideal environmental parameters were meticulously upheld, including a temperature of approximately 27 ± 1 °C, relative humidity at $65 \pm 5\%$, and a daily-adjusted light cycle of 10L:14D. Newly eclosed adults from this laboratory culture were exclusively utilized for the experiment, ensuring standardized and controlled conditions throughout the study. Different life stages of *Z. bicolorata* and its host plant are shown in Fig. 1.

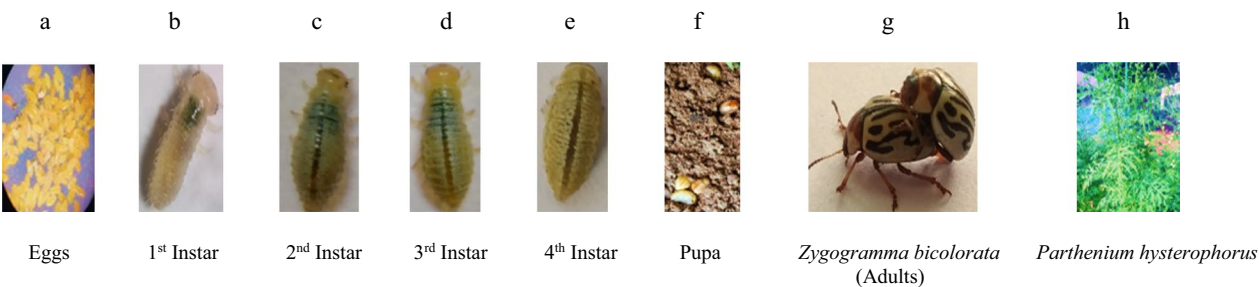


Fig. 1 Life stages of *Z. bicolorata* (a–g) and its host plant *P. hysterophorus* (h)

Reproductive and longevity assessment methodology

During present study methodology involved selecting six pairs of *Zygogramma bicolorata* from both the pendimethalin-treated and control groups, ensuring a balanced representation of one male and one female in each pair. These pairs were individually placed within separate petri dishes, facilitating focused observations. Throughout their entire oviposition period, we vigilantly tracked and recorded egg counts, enabling the accurate quantification of fecundity for each pair. Concurrently, we documented the specific oviposition days, enriching our understanding of their reproductive patterns. The longevity of male and female *Zygogramma* specimens was recorded, providing valuable insights into their lifespan dynamics. To evaluate egg viability and developmental studies, a pivotal aspect of our study, we pooled 180

eggs from the control and treated group each and further divided the 180 eggs into two batches, 150 eggs for studying egg viability and 30 for developmental studies as shown in Fig. 2.

This comprehensive approach not only captured the dynamics of fecundity, oviposition patterns, and longevity but also shed light on the crucial factor of egg viability. Such a methodology ensures a robust investigation into the impact of pendimethalin treatment on *Zygogramma* reproductive behaviour and overall reproductive success.

Methodology for assessing duration of developmental stages

To investigate immature development, we conducted a structured study. We formed three replicates for both treated and control groups, with each replicate

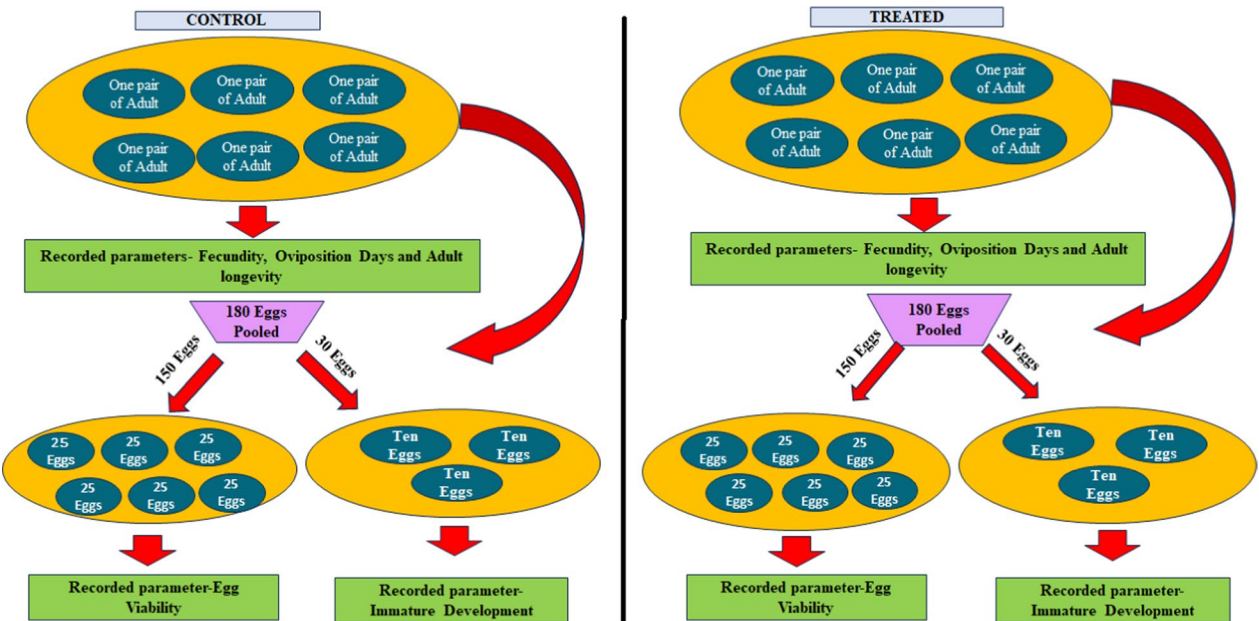


Fig. 2 Experimental setup for recording fecundity, oviposition days, adult longevity, egg viability, and immature development. Left panel, control and right panel, treated

containing 10 carefully selected eggs (Fig. 2). We strictly tracked the entire development process, noting each stage’s duration in both groups. This approach allowed us to compare how treatment might affect the developmental timeline of *Z. bicolorata* immatures.

Preparation of herbicide concentration

In this study, a commercial formulation of pendimethalin 30% EC, trade name pendamil, was used. Pendimethalin was used at a concentration of 0.71%, a choice that aligns with the recommended range defined by regulatory bodies in India as shown in Table 1. 0.71% concentration was taken to assess the potential impact of pendimethalin on non-target organisms, such as *Z. bicolorata*. The selected herbicide concentration was prepared through a process of dilution in distilled water, involving gradual dissolution and stirring for 30 min at room temperature. For the preparation of the selected concentration, 7.1 ml of pendimethalin was taken in a beaker and the volume was raised to 1L, thus giving us a solution of 0.71% concentration of pendimethalin (30% EC).

Bioassay

The bioassay was conducted using *Z. bicolorata* adults, totalling 300 individuals, which were divided into two groups: treatment (T) and control (C). The experiment was performed in triplicate, with each replicate of the T and C groups consisting of 50 *Z. bicolorata* individuals.

Concentrations of PND (0.71%) were prepared, and *Parthenium* leaves were exposed to PND using the leaf dip method (Qadri et al., 2022). The air-dried *Parthenium* leaves exposed to PND were subsequently provided to the T group. In contrast, the C group was fed air-dried *Parthenium* leaves that had been dipped in distilled water only.

Following the initial treatment with PND, both the T and C groups were subsequently supplied with fresh *Parthenium* leaves for the entirety of their lifespan. To assess the effects of PND treatment, samples were collected at various intervals, ranging from 24 to 120 h post-treatment. Specifically, at each sampling time point, three insects were randomly selected from each replicate within the T and C groups for biochemical analysis. Additionally, for genotoxicity assessment using the comet assay, three insects from each replicate of both

Table 1 Herbicide recommendations for approved crops

Approved crops	Pendimethalin 30% EC			
	Dosage /ha			Dilution in water (Litres)
	Weed species	a.i. (gm/ Kg)	Formulation in (gm/ ml / Kg/ ltr)	
Wheat	<i>Phalaris minor</i> , <i>Chenopodium album</i> , <i>Melilotus alba</i> , <i>Portulaca oleracea</i> , <i>Anagallis arvensis</i> , <i>Fumaria parviflora</i> , <i>Poa annua</i>	Light soil - 1.0 kg, medium soil - 1.25 kg, heavy soil - 1.5 kg	3.3 ltr 4.2 5.0	500–700 500–700 500–700
Rice	<i>Echinochloa colona</i> , <i>E. crusgalli</i> , <i>Fimbristylis miliacea</i> , <i>Marselia quadrifoliata</i> , <i>Alternanthera sessilis</i> , <i>Ammonia baccifera</i> , <i>Ludwigia parviflora</i> , <i>Eclipta alba</i> , <i>Cyperus difformis</i>	Light to Heavy soil 1–1.5 kg	3.3–5 Ltrs	500–700
Cotton	<i>Echinochloa spp.</i> , <i>Euphorbia hirta</i> , <i>Amaranthus viridis</i> , <i>Portulaca oleracea</i> , <i>Trianthema spp.</i> , <i>Eleusine indica</i>	0.75–1.25 kg	2.5–4.165 ltrs	500–700
Soybean	<i>Echinochloa spp.</i> , <i>Euphorbia spp.</i> , <i>Amaranthus viridis</i> , <i>Portulaca oleracea</i> , <i>Trianthema spp.</i> , <i>Eleusine indica</i>	0.75–1.0 kg	2.5–3.3 ltrs	500–700

the T and C groups were selected at specific time intervals following treatment, including 24 h, 72 h, and 120 h post-treatment.

Biochemical analysis

Preparation of tissue homogenates

The gut of *Z. bicolorata* was harvested at each sampling point and the foregut and hindgut were chopped off. The remaining midgut was used for the biochemical analysis. The midgut was cleaned and finely minced using a glass Teflon homogenizer. A 10% (w/v) tissue homogenate solution was made in a solution of 2 mM Tris-HCl and 50 mM mannitol buffer, pH 7.2.

Oxidative stress markers and antioxidant enzymes

All enzymatic and non-enzymatic oxidative parameters were measured in the tissue homogenate. Lowry method was used to determine the amount of protein in tissue homogenates (Lowry et al., 1951). The tissue homogenates were centrifuged at 5000 RPM for five minutes while kept at 4 °C after being deproteinized with the metaphosphoric acid precipitation technique. The concentration of reduced glutathione (GSH) was determined by assaying the supernatants with 5,5'-dithiobisnitrobenzoic acid (DTNB), as outlined by Beutler et al., (1963). Malondialdehyde's interaction with the dye thiobarbituric acid produces a pink complex (Buege & Aust, 1978) that absorbs light at 592 nm, and this complex was used to quantify lipid peroxidation (LPO). Aebi's method for determining the conversion of H₂O₂ to water and oxygen dioxide at a wavelength of 240 nm is commonly used to assess the enzyme catalase's (CAT) activity (1984). The activity of superoxide dismutase (SOD) was determined by observing the inhibition of the auto-oxidation of pyrogallol, as described by Marklund and Marklund (1974). The assay for glutathione peroxidase (GPx) involved measuring the conversion of NADPH to NADP+ as a decrease in absorbance at 340 nm, as described by Flohe and Gunzler (1984).

Comet assay - single-cell gel electrophoresis

A modified version of the comet assay method described by Singh et al. (1988) was used to analyse DNA damage. A single-cell suspension of the midgut was prepared in RPMI medium and combined with 1% low-melting agarose, and the mixture was then layered onto agarose-coated slides that had been chilled at 4 °C for 30 min. The slides were then incubated at 4 °C for another 30 min to solidify after the addition of another layer of agarose. The remaining steps followed by the protocol described by Maheshwari and Mahmood (2022).

To evaluate DNA damage, the stained slides were examined using a CX41 fluorescent microscope

(Olympus, Japan) at a magnification of 100X. The length of the comet tail was measured in photographs using Komet 5.5 software (USA) as an indicator of DNA damage. Duplicate slides of each sample were analysed, and the image analysis system automatically calculated the comet tail length for 50 cells from each slide. The CX41 fluorescent microscope was used to score the cells for DNA damage after staining with ethidium bromide.

Statistical analysis

The statistical analysis was conducted to assess the significant difference between the control (C) and treatment (T) groups across various response variables, including fecundity, egg viability, oviposition days, longevity, biochemical parameters (oxidative stress markers and antioxidant enzymes), and DNA damage assessed through the comet assay. One-way analysis of variance (ANOVA) was utilized to determine if statistically significant differences existed between the groups for these response variables, with significance levels set at $p < 0.05$ and $p < 0.01$ for highly significant results. Post hoc Tukey's tests were applied following ANOVA when significant differences were identified. All statistical analyses were conducted using GraphPad Prism 5, providing a comprehensive assessment of the impact of pendimethalin on *Z. bicolorata* across a range of parameters.

Results

Biological attributes

Mortality

The mortality rates exhibited consistent and minimal variations during the 120-h experimental duration, as outlined in Table 2.

Reproductive and longevity assessment

The experimental results demonstrated significant impacts on the reproductive and longevity parameters of *Z. bicolorata* due to pendimethalin exposure. In the

Table 2 Corrected mortality (%) of *Z. bicolorata* after treatment with pendimethalin (dose = within min. field-recommended dose) recorded for a duration of 120 h post-treatment

Corrected mortality (%) of adult of <i>Z. bicolorata</i>	
Time (h)	CM (%)
24	5.33 ± 1.15
48	2.09 ± 2.08
72	0 ± 0.00
96	0 ± 0.00
120	0.70 ± 1.22

CM: Corrected mortality using Abbott's formula (Abbott, 1925)

treated group compared to the control group, fecundity exhibited a significant decrease of approximately 20.98%. Similarly, egg viability percentage showed a significant decline of about 18.36%. Although there was a noticeable impact on adult longevity, with male longevity significantly decreasing by approximately 13.80% and female longevity by about 11.18%, oviposition days were not significantly different, with only an insignificant decrease of approximately 9.18% observed in the treated group ($p > 0.05$), as shown in Fig. 3.

Effect on developmental stages

The impact of pendimethalin also extended to the developmental stages of *Z. bicolorata*. The incubation period of eggs increased significantly by approximately 19.85% ($p < 0.05$). While the duration of the I instar was slightly prolonged ($p > 0.05$), the II instar duration showed a significant extension of approximately 16.39% ($p < 0.05$). Moreover, the treated group exhibited a substantial increase in the duration of the III instar by approximately 18.25% ($p < 0.05$), and the duration of the IV instar increased by approximately 23.27% ($p < 0.05$). The pre-pupa stage was also significantly impacted, with its duration increasing by about 35.78% ($p < 0.05$). Furthermore, the duration of the pupa stage increased by approximately 14.52% ($p < 0.05$), as shown in Fig. 4.

These findings underscore the substantial adverse effects of pendimethalin on the developmental stages and reproductive biology of *Z. bicolorata*, emphasizing the potential ecological consequences of herbicide use on non-target organisms.

Biochemical analysis

The treatment of PND caused the levels of enzymatic and non-enzymatic parameters of oxidative stress in the gut of *Z. bicolorata* to plummet. GSH level was decreased, and at the sampling point of 120 h post-treatment with PND, the remaining level was 48.81% of untreated control insects (Fig. 5a). The treatment of PND also led to lipid peroxidation in the midgut tissue. The greatest increase of 1.78 fold with respect to control values was seen at the sampling point of 120 h post-treatment (Fig. 5b).

The GPx activity was decreased in the PND-treated groups, and the decrease in GPx activity continued and was 50.8% of control insects, at the last sampling point of 120 h (Fig. 5c). PND treatment also reduced the activity of SOD and continued to a downward trend till the 120 h sampling point. The most severe decrease of 52% was seen at the sampling point of 120 h (Fig. 5d). A statistically significant decrease in the CAT activity was also seen in PND-treated groups and continued to decline

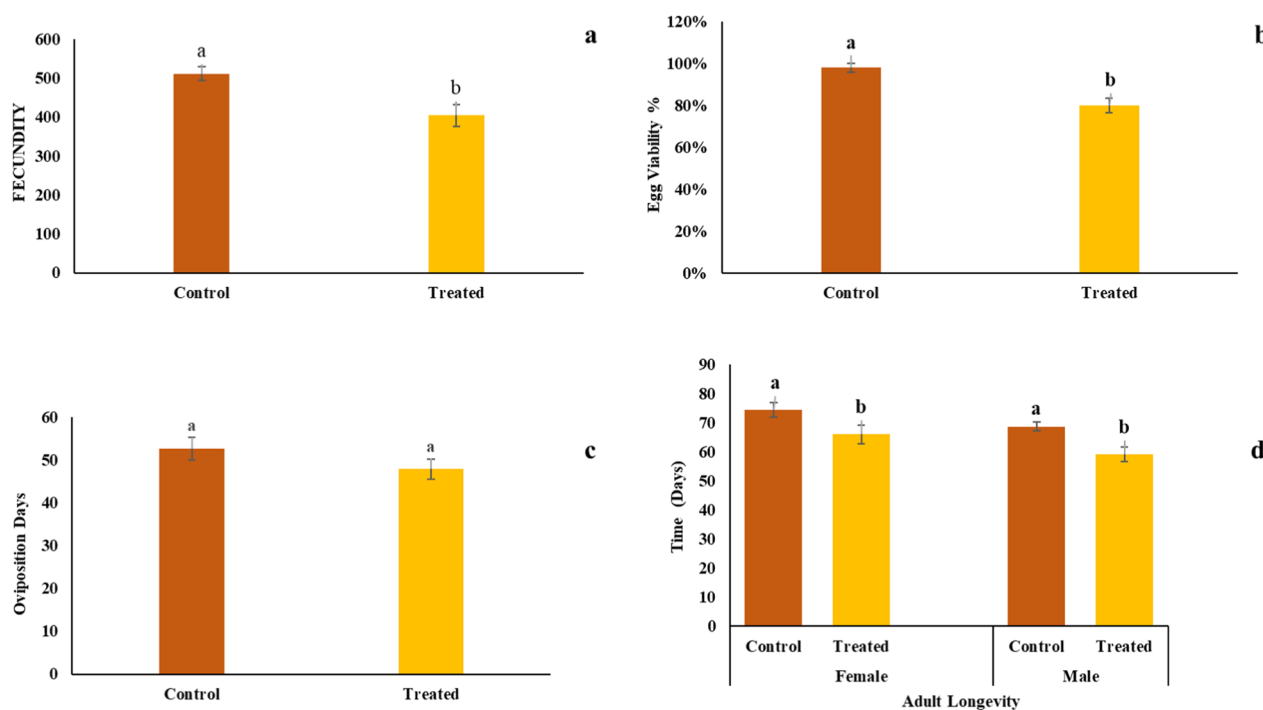


Fig. 3 Impact of Pendimethalin Exposure on Reproductive Performance and Adult Longevity of *Z. bicolorata*. **a** (Fecundity), **b** (Egg Viability Percentage), **c** (Oviposition Days), and **d** (Adult Longevity) present distinct components of this investigation. Groups labelled with different alphabets are statistically significant at $p < 0.05$ compared to the control group, and with same alphabets are statistically insignificant ($p > 0.05$)

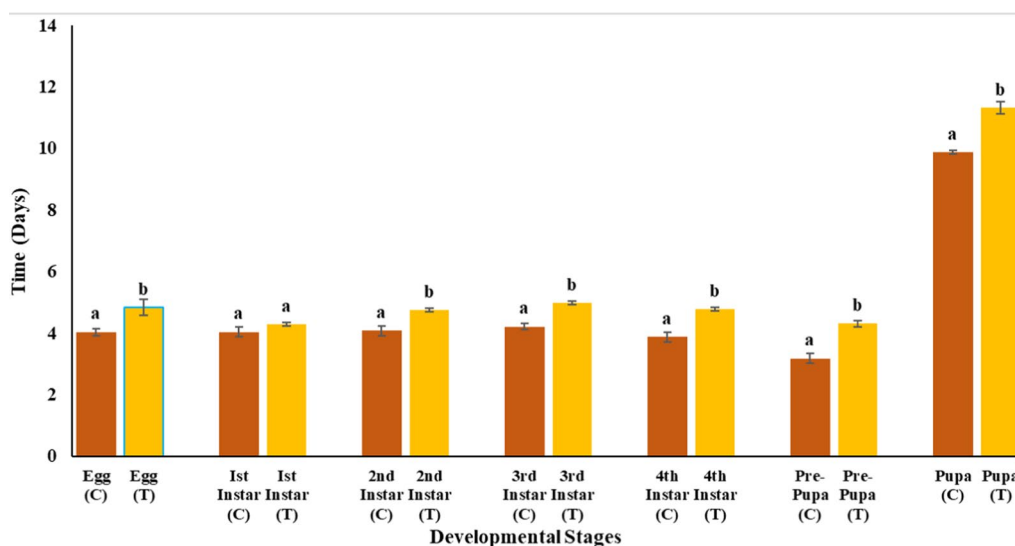


Fig. 4 Stage-specific and total immature development of *Z. bicolorata* under the influence of pendimethalin. Groups with different alphabets are significantly different at $p < 0.05$ from control, and with same alphabets are statistically insignificant ($p > 0.05$)

till 120 h (47% of control values), the last sampling point (Fig. 5e).

Comet assay

Single-cell gel electrophoresis (comet assay), a highly sensitive technique, was employed to assess DNA damage caused by PND (30% EC) post-treatment to the midgut at specific time intervals, including 24 h, 72 h, and 120 h. The comet images from the midgut of control insects (Fig. 6a) and PND-treated insects (Fig. 6b–d) revealed DNA degradation, as evidenced by an increase in DNA tail length (Table 3).

Discussion

Pesticides are necessary for agriculture to prevent the infestation of insect pests, viruses, and undesirable plants that can infiltrate and damage crops. However, in addition to harming the targets, these chemicals also affect organisms that are not targets (Pasini et al., 2021; Serrao et al., 2022). The use of herbicides in agriculture has a long-lasting detrimental impact on non-target organisms that inhabit or frequent the exposed areas (Marcato et al., 2017; Mauser et al., 2023). Non-target species may be exposed to pesticides through normal behaviours like flight or movement in search of food, shelter, and resting on plants treated with insecticides (Laycock et al., 2012; Pathan et al., 2008).

We conducted this study to evaluate the effects of the minimum recommended field doses of herbicide PND on life metrics, biochemistry, and DNA of a bio-control beetle *Z. bicolorata* under lab conditions.

Pesticides have been shown to significantly increase oxidative stress in various animal species, including insects (Ashraf et al., 2022). Oxidative stress results from the imbalance in the equilibrium between reactive oxygen species (ROS) production and their scavenging, thus shifting it towards more ROS production, overtaxing the organism's natural anti-oxidative defence system (Lukaszewicz-Hussain, 2010; Nishida, 2011). In our study, at the biochemical level, the treatment of PND led to a decrease in the levels of major intracellular antioxidant GSH in the midgut of *Z. bicolorata*. Lowered GSH level accelerates the production of reactive oxygen species (ROS), leading to oxidative damage (Sule et al., 2022). Oxidative stress has a strong effect on the development of some pests like *Helicoverpa armigera* (Apirajkamol, et al., 2020), *Spodoptera littoralis* (Hamama & Fergani, 2019), *Galleria mellonella* (Buyukguzel & Kalender, 2009). The generation of ROS causes oxidation of polyunsaturated fatty acids (PUFAs) in the cell membrane to produce malondialdehyde (MDA). MDA is a potent marker of lipid peroxidation (LPO). In our study, PND increased the MDA level in the midgut of *Z. bicolorata*, making the gut cell membrane more fragile and, therefore, might have caused damage to the cellular components. Some earlier research in honey bees supports our approach since PND causes oxidative stress in the non-target organism, *Z. bicolorata*, in our investigation (Chakrabarti Basu et al., 2014; Pal et al., 2022). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are the primary antioxidant enzymes of antioxidant defences. SOD converts superoxide radicals to hydrogen peroxide (H_2O_2), a non-radical ROS

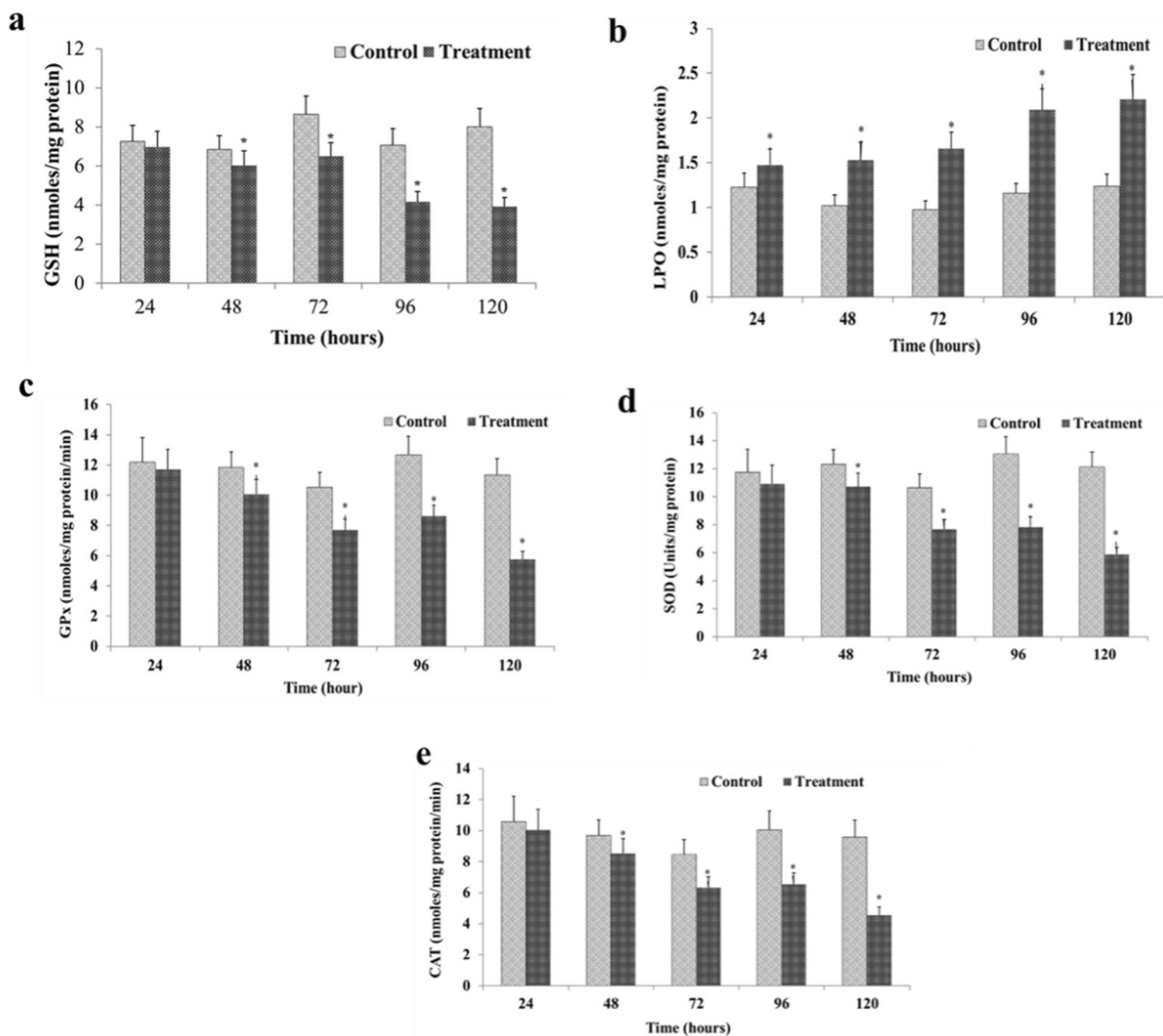


Fig. 5 All the non-enzymatic and enzymatic parameters of oxidative stress were determined in tissue homogenates prepared from midgut of *Z. bicolorata*. **a** GSH, **b** LPO, **c** GPx, **d** SOD, and **e** CAT from 24–120 h post-treatment with pendimethalin to the adult *Z. bicolorata*

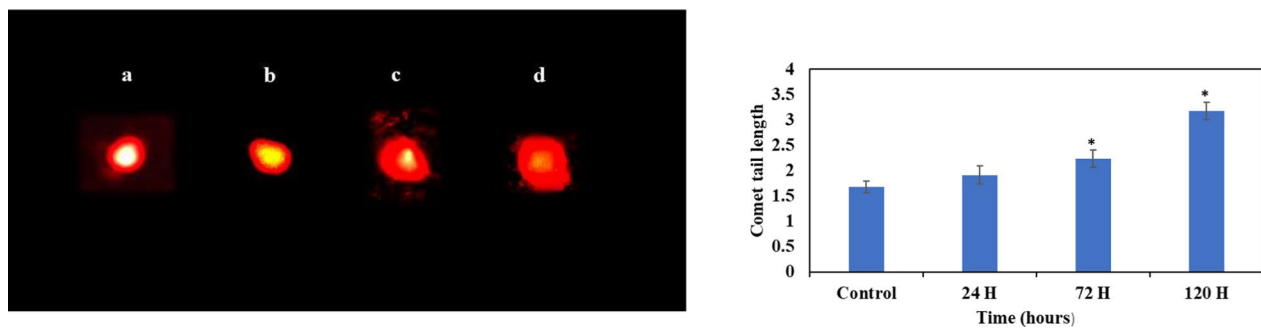


Fig. 6 Single-cell gel electrophoresis (comet assay) of midgut of the *Z. bicolorata* from **a** control, **b** 24H, **c** 72H and **d** 120 h of post-treatment with herbicide pendimethalin at minimum field-recommended dose. *Significantly different at $p < 0.05$ from control by one-way ANOVA using post hoc Tukey's test

Table 3 Comet tail length of midgut of adult *Z. bicolorata* after treatment with pendimethalin

Comet tail length	Control	24 h	72 h	120 h
Gut	1.68 ± 0.12	1.91 ± 0.18	2.24 ± 0.17*	3.18 ± 0.17*

Results are mean ± standard error of five different preparations

*Significantly different at $p < 0.05$ from control by one-way ANOVA using post hoc Tukey's test

followed by decomposition of hydrogen peroxide to non-toxic water and molecular oxygen by catalase. The inhibition of SOD and CAT will produce excessive H_2O_2 and superoxide radicals. GPx is a detoxifying enzyme that initiates the conjugation of glutathione with harmful substances. It also helps in the dissociation of H_2O_2 to non-reactive species. Inhibition of antioxidant enzymes in the midgut of *Z. bicolorata* will increase the generation of ROS. ROS further damages the cell and cellular components like thiols, proteins, lipids, and DNA. Thus these results suggest that reduced thiol status, increased LPO, and compromised antioxidant enzymes will cause oxidative damage to gut cell in *Z. bicolorata*, which may lead to an increased mortality rate in insects.

The comet assay or single-cell gel electrophoresis is highly sensitive and versatile technique to evaluate DNA damage. Administration of PND to *Z. bicolorata* caused DNA damage in gut cells which is evident by increased comet tail with respect to the control (Patel et al., 2007). PND induced genotoxicity by either ROS generation or interaction of PND or its metabolites directly with cellular DNA, ultimately leading to double-strand breaks in DNA. The ROS-induced DNA damage has also been seen in other insects like silkworm, where DNA breaks were caused by the pesticide-induced ROS stress (Qadri et al., 2022).

Our findings show that herbicide PND adversely affects the physiological characteristics of adult *Z. bicolorata* at a minimum field-recommended dosage. It was also observed that the use of PND had a negative impact on *Z. bicolorata*, on account of mortality rate, prolonged immature development, reduced fecundity, decreased egg viability, decreased oviposition days, and reduced adult longevity, thus affecting it both directly and indirectly. These findings are consistent with research that found adverse effects of herbicides on biological control agents at various exposure levels *Neochetina eichhorniae* and *Eccritotarsus catarinensis* (Hill et al., 2012). The current study also showed that PND treatment lowered *Z. bicolorata* reproductive characteristics, including fecundity and oviposition days. These results are supported by earlier research on *Z. bicolorata* (Hasan & Ansari, 2016). In the PND-treated *Z. bicolorata* group, the immature stage development took a noticeably longer duration

than the control. The prolonging of development time in our study may be partly explained by increased oxidative stress in the adult. These results corroborate earlier studies in *Bracon hebetor* (Muslim et al., 2018).

The study showed the dangers of PND use even in minimum recommended doses to the non-target organism and an important bio-control agent *Z. bicolorata*, affecting its life parameters and biology. To prevent this damage to the non-target organism, it is necessary to find ways where we will be able to save non-target organism in order to preserve biodiversity and the ecosystem and also to protect crops from damage from weeds.

Conclusion

Our research highlights the significant risks posed by the herbicide pendimethalin to the non-target beetle *Z. bicolorata*, a critical bio-control agent for managing the invasive weed *P. hysterophorus*. Even at the minimum recommended field dose, pendimethalin causes gut damage, oxidative stress, and DNA damage in these beetles. Responsible pendimethalin application in agriculture is crucial to protect *Z. bicolorata* and maintain a balance between farming productivity and ecological sustainability. This underscores the need for a more thoughtful approach to pest management, ensuring the long-term health of our agricultural systems and the preservation of biodiversity.

Abbreviation

PND Pendimethalin 30% EC

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Author contributions

NS and AQ contributed to conceptualization and formal analysis and investigation; NS contributed to methodology and writing-original draft preparation; NS, AQ, and NM contributed to resources and review and editing; and AQ supervised the study.

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Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study needed no ethical approval. The study involves no human trial.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing or financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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