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# EFFECTS OF HERBICIDE GLYPHOSATE ON FITNESS ATTRIBUTES OF *ZYGOGRAMMA BICOLORATA* PALLISTER

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**ABSTRACT**

**This study is on the effects of glyphosate on biology and fitness of biocontrol agent *Zygogramma bicolorata* against parthenium weed. Bioassay of glyphosate was conducted using a minimum range of the field-recommended dose (0.408%). Results showed that indirect exposure to glyphosate caused significant mortality of I, II, III, and IV instar *Z. bicolorata* grubs. Maximum mortality (23.60%) was recorded in the first instar. The total larval developmental period was significantly prolonged in the group exposed to glyphosate (19.50 days) compared to the unexposed group (17.73 days). The application of glyphosate also caused a variation in pupation period. In addition, the longevity of both male and female *Z. bicolorata* was negatively impacted by glyphosate treatment. Thus, it is concluded that glyphosate has an ecotoxic effect on *Z. bicolorata* with potential implications for the effectiveness of *Z. bicolorata* as a biocontrol agent against parthenium.**

**Key words:** Bioassay, biocontrol, ecotoxic, fitness, glyphosate, mortality, parthenium, *Zygogramma bicolorata*, larva, pupa, adults, longevity, mortality, development fecundity

Carrot grass, scientifically named *Parthenium hysterophorus* L., is a widely recognized and problematic weed within the Asteraceae family. This weed is notorious for causing numerous health issues in both humans and animals, as well as negatively impacting crop productivity and biodiversity (Kumar et al., 2012). In India, parthenium weed made its first presence in Pune during 1955 (Rao, 1956) and currently occupying an area of 35 million ha in India (Singh et al., 2008). Several efforts were made to effectively bring this weed under control. A leaf feeding host-specific *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) has been found to be the most effective as a biological control agents. This beetle was introduced from Mexico to India by the Indian Institute of Horticultural Research, Bangalore (Jayanth, 1987), and became abundant within three years (Jayanth and Visalakshy, 1996). The augmentative release of *Z. bicolorata* was made in India during 1984 and in Jammu and Kashmir in 1989.

The integration and simultaneous use of herbicides and biocontrol agents could be an effective IPM strategy. However, this approach could only be accomplished when applied herbicides are non-toxic to the biological control agent. Toxicological studies were used previously to assess the toxicity of synthetic chemicals on biocontrol agents. Jayanth and Bali (1993) reported the lethal effect on *Z. bicolorata* by commonly used herbicides. Less literature is available to demonstrate the negative effects of glyphosate on biology attributes of insects as well as on their ecology. Contrary results on the non-target and target effects of herbicides exist in literature (Schneider et al., 2009; Benamu et al., 2010). Herbicide affects development of insects and it will also exert negative effect on biological attributes of *Z. bicolorata*. Hence, a study was carried out at the laboratory, Division of Entomology, FoA, SKUAST-Jammu, Chatha, India, to evaluate the detrimental effects of glyphosate on biological attributes viz., development and fertility of *Z. bicolorata.*

## MATERIALS AND METHODS

*Zygogramma bicolorata* adults were collected from the field and reared in the laboratory on parthenium leaves and stems. The grubs were transferred to fresh vials as they developed. Pupation occurred in soil-filled glass jars, and the hatched adults were provided with parthenium leaves for nourishment and oviposition. Glyphosate herbicide (Roundup, 41% SL) that is used to control parthenium weed, was evaluated against *Z. bicolorata* using the lowest recommended dose (0.408%). Parthenium leaves and twigs were dipped in

0.44% glyphosate solution and air-dried. The control group received sterile distilled water. Glyphosate-treated leaves were placed in vials and kept in glass jars; *Z. bicolorata* grubs were introduced into the jars and replicated 15 times. The jars were incubated at 26±2°C, RH of 66±7% for 15 days. Glyphosate-treated leaves were replaced after 24 hr in the bioassay. The mortality of treated larvae was measured, reflecting larval mortality within 24 hr. Larvae killed were counted and excluded. Fully developed larvae were transferred to sterilized jars for pupation, and observations were made on mortality, larval development period, and pupation (Hasan and Ansari, 2016). Third instars larvae treated with glyphosate in direct exposure were obtained and kept in petri plates containing fresh leaves of parthenium.

Daily visual observation for hatching of eggs and newly developed larvae were made. Determination of instars mortality was assessed by live and dead larvae observing the exuviae or head capsule. The prepupae were transferred moist sterilized sand filled in glass vials. Development time of larval stages, prepupae, and pupae was determined in treated and control. Each treatment was replicated 15 times. Emerged adults obtained from above experiment were grouped. Each pair of male and female was kept in glass vials (10 pairs replicated 15 times). Each pair was fed with leaves of parthenium for oviposition. Whenever a male died, new male was introduced and thus each female had only single male available for mating. Daily observations on eggs laid by female from the first day of oviposition until death of each female was recorded. In order to get the total number of female births, eggs were divided on base of sex ration of 1:1 ratio. Other parameters viz., preoviposition, oviposition adult emergence, postoviposition of newly emerged adults and egg viability was also observed. The data was subjected to statistical analysis, through SPSS software.

## RESULTS AND DISCUSSION

The direct effect of glyphosate on the mortality rate (%) of *Z. bicolorata* was assessed (Fig. 1). The findings revealed a significant mortality rate in instar grubs I, II, III, and IV. These data demonstrate the adverse impact of glyphosate on the survival of *Z. bicolorata*. Early instars were highly susceptible to the doses of glyphosate than the latter; maximum mortality (23.60%) was recorded in I instar, followed by II (20.80%) and III (18.80%). However, least mortality of 15.40% was recorded in IV instar. Pupation

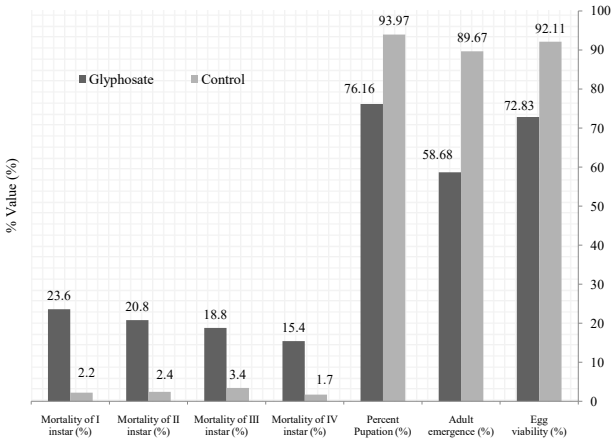


Fig. 1. Effects of glyphosate herbicide on mortality and reproduction of *Z. bicolorata*

was also affected (76.16%) as against 93.97% in control. The minimum (58.68%) adult emergence was observed in grubs treated with glyphosate as against 89.67% in control. Mean egg viability of control batch was 92.11% which decreased to 72.83% with the application of glyphosate. Thus, it was observed that glyphosate caused ecotoxic effects on biology of *Z. bicolorata*. Adverse effects on biological attributes of insects caused by the application of herbicides are limited (Benamu et al., 2010; Marambe and Herath 2020; Halangoda et al., 2022).

The study also investigated the indirect effect of glyphosate herbicide on the fitness attributes of *Z. bicolorata*. Results (Fig. 2). The results revealed that glyphosate application significantly increased the developmental period of instars I, II, III, and IV by 5.44, 4.60, 4.54, and 4.92 days, respectively, compared to the control periods of 5.17, 4.17, 4.10, and 4.29 days. These data indicate a negative impact on the fitness attributes development, and may compromise their effectiveness as a biocontrol agent for parthenium weed. Herbicidal treatment of glyphosate greatly affected total larval

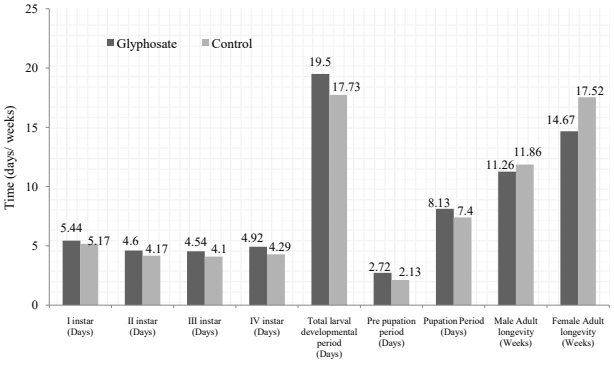


Fig. 2. Effects of glyphosate on biology of *Z. bicolorata*

development period of immatures of *Z. bicolorata*. A total of 19.50 days of larval period was recorded when the grubs were exposed to glyphosate which was found significantly prolonged when compared to the non-treated grubs (17.73 days). The mean values of prepupation development period increased with the application of herbicide. The average values of control and glyphosate treatments on the prepupation period were 2.13 and 3.49 days, respectively. Variation in pupation period was also observed. Sex ratio (proportion of female) was considerably higher in the grubs treated with glyphosate. Herbicide treatment caused a negative impact on the longevity of male and female. Mean longevity of 17.52 and 14.67 weeks, was recorded in females of control and glyphosate batch, respectively. Mean longevity of control males were 11.86 which decreased to 11.26 weeks with the application of glyphosate. Maximum dosage of herbicides significantly prolonged the duration of *T. evanescens* development (Sebai et al., 2012). The present findings are also in agreement with those on the adverse effect on developmental time of biocontrol agents of water hyacinth (Jianqing et al., 1998). The possible effect of prolonged larval development of *Z. bicolorata* may be due to the indirect effect of herbicides caused on insects by altering the plant growth or destroy the food supplies, which were less favourable to larval development of *Z. bicolorata* (Hasan and Ansari, 2017).

The indirect effects of glyphosate on the reproductive attributes of *Z. bicolorata* when it was found that females showed a significantly higher preoviposition period compared to the control group. Thus there exists a negative impact on the reproductive abilities of *Z. bicolorata*. Preoviposition period was recorded higher by glyphosate (7.20 days) treatment as compared to control (5.76 days) (Fig. 3); and oviposition period decreased 71.33 days) as against 102.83 days. Females when exposed to glyphosate exhibited lower post-oviposition period of 10.83 days, Post-oviposition period was also significantly effected (12.00) days. Glyphosate caused negative impacts on fecundity in control it was (1730.7 as against 731.67). Numerous studies have also demonstrated less adverse glyphosate treatment effect on insect herbivores which are used as biological control of several terrestrial weeds (Nelson and Lym, 2003). Herbicides cause numerous sublethal effects such as, prolonged development (Schneider et al., 2009), reduced fecundity and alteration in fertility rates (Paoletti and Pimentel, 2000), and fluctuations in oviposition behaviour (Schneider et al., 2009). Harmful effects on biocontrol agents with exposure levels of herbicide had been reported (Osama et al., 2012; Mao et al., 2021).

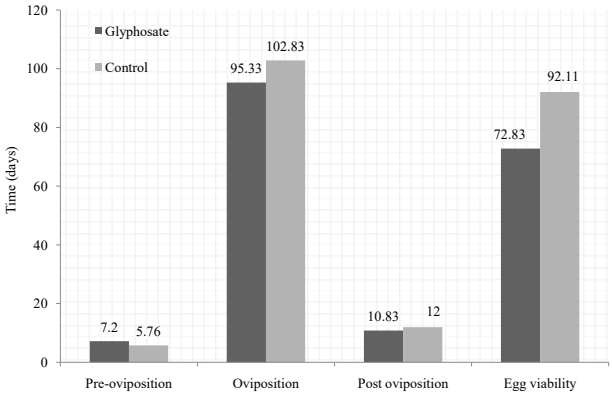


Fig. 3. Effects of glyphosate herbicides on reproductive fitness of *Z. bicolorata*

Thus glyphosate induces a range of direct and indirect effects on biological attributes of *Z. bicolorata*, even in minimum recommended field dose. Thus, it can be concluded that suitable selection of right pesticides and accurate dosage would be a crucial factor for maintaining the natural populations of *Z. bicolorata.* Based on this study, it is noteworthy that the beetles that are able to withstand glyphosate application may potentially develop into resistant variants. The emergence of glyphosate-resistant *Z. bicolorata* beetles despite the toxicity may present a promising opportunity for integrated weed management efforts. By incorporating these resistant beetles with other weed control methods, it may be possible to be more effectively manage parthenium.

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**AUTHOR CONTRBUTION STATEMENT**

Conceptualization: SA, RKG; Data: SA, RKG, SAA; Formal analysis: SA, RKG, UB, RSB, SKS, SAA; Writing -original draft: SA, UB, DP, SAA; editing: UB, DP, SAA. All authors read and approved the manuscript.

**CONFLICT OF INTEREST**

No conflict of interest.

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