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Research Article

Evaluation of host-specificity of *Zygogramma bicolorata* Pallister (Coleoptra: Chrysomelidae) for the biological control of *Parthenium hysterophorus* L. (Asteraceae: Heliantheae) in Nepal

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ABSTRACT

Host-specificity test of *Zygogramma bicolorata* Pallister (Coleoptra: Chrysomelidae) was conducted in the field and laboratory of National Entomology Research Center, Khumaltar, Lalitpur, Nepal during April to September, 2017. Multiple-choice and no-choice tests were conducted on *Gerataum houstoniamum* Mill., *Bidens pilosa* L., *Chrysanthemum indicum* L., *Dahlia pinnata* Cav, *Guizotia abyssinica* L., *Helianthus annuus* L., *Lactuca sativa* L., *Parthenium hysterophorus* L., *Perilla frutescence* L., *Xanthium strumarium* L., *Zinnia elegans* Jacq. and *Jasminum officinale* L. Among tested plant species, *P. hysterophorus* was only a preferred host of *Z. bicolorata* on which both larvae and adults fed. Ovipostion, larval development, pupation and adult emergence of *Z. bicolorata* occurred successfully on *P. hysterophorus* completing its life cycle. Larvae consumed *H. anuus* but could not pupate, and adults fed on it when starved for 5 days in no-choice test. Both adults and larvae of *Z. bicolorata* consumed *X. strumarium* and completed larval and pupal developments, but adults did not oviposit. Adult longevity was significantly reduced after feeding on *H. annus* (19.00 days) and *X. strumarium* (29.33 days) compared to *P. hysterophorus* (83.33 days).

Keywords: *Z. bicolorata*, host-specificity, multiple-choice, no-choice, adult longevity, oviposition.

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INTRODUCTION

Parthenium hysterophorus L. (Asteraceae; Heliantheae), commonly known as parthenium, is an annual or short-lived perennial herbaceous plant. It is regarded as one of the worst weed currently known due to its invasiveness, potential for spread, impact upon human health and economic and environmental impact (Adkins & Shabbir, 2014). This weed has characteristics of prolific nature producing up to 25000 viable seeds per plant, allelopathic potential, high tolerance to abiotic stresses; phentotypic plasticity in its growth form, ability to grow in wide

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range of edaphic conditions and also exhibits C3/C4 phtosynthetic mechanism (Shrestha *et al*., 2019). Parthenium is native to region surrounding the Gulf of Mexico including southern United States, northern Argentina, southern Bolivia and south-west Brazil (Navie *et al*., 1996). The weed has spread into 96 countries of Africa, Asia, Europe, North America, Ocenia and South America (CABI, 2020). The first specimen of *P. hysterophorus* was collected from the Trishuli Valley of Nepal in 1967 (Tiwari *et al*., 2005). Parthenium was reported as dominant weed species from Kathmandu, Hetauda, Bharatpur, Butwal, Pokhara Dang, Surkhet, and Nepalgunj of Nepal (Shrestha *et al*., 2014). Parthenium hysterophorus is one of the invasive plant species in Nepal and is one of the threats to agriculture, resulting in crop loss and increased production cost (Shah *et al*., 2020). It can be managed through physical, chemical and biological management approaches. Insects have received maximum attention as sustainable biological control of parthenium (Sushilkumar, 2009). Biological control of parthenium initiated from Australia in 1977 and leaf feeding beetle, *Zygogramma bicoloata* Pallister (Coleoptra: Chrysomelidae) was introduced from Mexico in 1980 (McFadyen and McClay, 1981). Biological management of parthenium weed initiated in India with introduction of *Z. bicolorata* in 1984 (Dhileepan and Strathie, 2009). There was no record of deliberate introduction and release of *Z. bicolorata* in Nepal but fortuitously arrived from India and has been found in various parts of Nepal (Shrestha *et al*., 2010: Shrestha *et al*., 2011).
Classical biological control and augmentative biological control are two main strategies of biological control of weed. Classical biological control refers selection of natural enemies of invasive species in their native place and releasing them in invaded environment expecting to establish, spread and self-sustaining effect on the target species. Augmentative biological control involves mass production and release of indigenous or exotic natural enemies into various environments expecting rapidly control of target species, but do not persist over long period of time in the released environment. Host-specificity has significant importance in both categories of biological control of pest. Host-specificity appears to be one of the most variable biological trait of natural enemies showing different degrees of specificity, from organisms having a narrow host range restricted to a species or a genus to those with a wide spectrum of potential hosts covering several orders, classes and even kingdoms. Generalist species of natural enemies are capable of exploiting various resources and switching from one host species to another as competition increases or host condition deteriorates while specialist species of natural enemies establish stronger links with their host (Brodeur, 2012).

Host-specificity of natural enemies is fundamental for success of biological control (Waage, 2001) and it affects effectiveness of natural enemy against the target species. Natural enemy with wide host range might have risk on non-target species, whereas, narrow host specificity could be a limiting factor for the commercialization of natural enemy (Brodeur, 2012). Host specificity test of bio-control agents of weeds are conducted on many aspects of the biology of agents including oviposition, adult feeding, larval feeding, larval development, adult longevity and fecundity. Various test designs are available and performed on the basis of suitability which depends on the biology of the potential agent being tested and choice and no-choice tests are the commonly used designs (Heard, 2002). Considering all these facts host-specificity of *Z. bicolorata*, an herbivore biological control agent of *P. hysterophorus* was studied with multiple-choice and no-choice tests in the National Entomology Research Center of Nepal Agricultural Research Council and findings are presented in this paper.

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METHODOLOGY

Host-specificity test of *Z. bicolorata* was conducted in the field and laboratory of National Entomology Research Center, Khumaltar, Lalitpur, Nepal during April to September, 2017. Multiple-choice and no-choice tests were conducted on 11 different plant species of Asteraceae family and one plant species of Oleaceae family (Table 1). Plant species were selected on the basis of previous research findings about possible host plant species of *Z. bicolorata* and taxonomic relationship of test plant with *P. hysterophorus*. Both cultivated and wild plant species were included in host-specificity tests.

Table 1: Plant species included in host-specificity tests of *Z. bicolorata*

S. N.	Plant species	Family	Common name
1	*Agerataum houstoniamum* Mill.	Asteraceae	Blueweed
2	*Bidens pilosa* L.	Asteraceae	Black-jack
3	*Chrysanthemum indicum* L.	Asteraceae	Chrysanthemum
4	*Dahlia pinnata* Cav	Asteraceae	Dahlia
5	*Guizotia abyssinica* L.	Asteraceae	Niger
6	*Helianthus annuus* L.	Asteraceae	Sunflower
7	*Lactuca sativa* L.	Asteraceae	Lettuce
8	*Parthenium hysterophorus* L.	Asteraceae	Parthenium
9	*Perilla frutescence* L.	Asteraceae	Perilla
10	*Xanthium strumarium* L.	Asteraceae	Cocklebur
11	*Zinnia elegans* Jacq.	Asteraceae	Common zinnia
12	*Jasminum officinale* L.	Oleaceae	Jasmine

Culture and mass production of *Z. bicolorata*

Initial culture of adult *Z. bicolorata* was collected from banks of Khageri river (N 27º34.110', E 84º43.792') in Chitwan district of Nepal. This insect was reared in semi-transparent rectangular plastic boxes of size 22.7 cm X 16.3 cm X 9 cm, length, breadth and height with volume of 1150 ml. Ventilation was provided in rearing box through nylon net window of 10 cm X 5 cm. Bouquets of fresh parthenium leaves mixed with flowers were provided as food for adults as well as larvae of *Z bicolorata*. Petioles of leaves were covered with moistened cotton and wrapped with aluminum foil in order to prevent quick drying of leaves. The bouquets of leaves were changed daily in rearing boxes. Eggs laid were collected with the help of camel hair brush and used for multiplication of the insect. The eggs of the insect were kept in 9 cm petri plate for hatching and first instar larvae after hatch were transferred into rearing boxes along with bouquets of parthenium leaves. Pre-pupae were transferred into pupation boxes containing 5 cm high soil as substrate. Substrate for pupation was prepared from field collected soil and sand mixed in 1:1 ratio. Substrate was sterilized in hot air oven at 80°C for 30 minutes and distilled water was sprinkled with hand sprayer to maintain moisture in soil. Newly emerged adults of *Z bicolorata* were again transferred into plastic rearing cages with bouquets of parthenium leaves for mating and further mass multiplication. Adults and larvae produced in laboratory cultures were used in the host-specificity studies.

Multiple-choice test

Multiple-choice test was conducted to determine host specificity of *Z. bicolorata* with 11 plant species of family Asteraceae (S. N. 1-11 from Table 1). Test was conducted inside the screen house in the fields of National Entomology Research Center with three replications during April - June, 2017. Plot size of 3.3 m X 1.6 m was used for each replication. Plants of *Guizotia abyssinica* L., *Helianthus annuus* L., *Lactuca sativa* L. and *Perilla frutescence* L. were prepared in the nursery of National Entomology Research Center. Similarly, plants of *Chrysanthemum indicum* L, *Dahlia pinnata* Cav., and *Zinnia elegans* Jacq. were purchased

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from local flower nursery. Whereas, seedling of weed plants, *Agerataum houstoniamum* Mill., *Bidens pilosa* L., *Parthenium hysterophorus* L. and *Xanthium strumarium* L. were collected from surrounding fields. Land was prepared and 110 kg of good quality compost was added in each replication. Four plants of each test species were planted in a single row at the spacing of 30 cm X 40 cm. 21- 30 days old seedlings were transplanted in randomized rows within each replication and plants were irrigated regularly with watering can.

Each replication was covered around with nylon screen before release of *Z. bicolorata* in order to prevent movement of the test insect (Figure 1a). One week old adults of *Z. bicolorata* without sexing were released, 15 days after transplanting of the plant species. A total of 100 adults were released inside each replication dividing into four equal groups and placed in four corners on the petri plates. Number of adult *Z. bicolorata* moved on different plant species after four hours was counted. Similarly, adult beetle population on different plant species after one, two, seven, 14 and 21 days of release were counted. Oviposition was observed on test plant species and number of eggs after two, four, seven and 14 days of adult insect release were counted. Similarly, number of larvae after seven and 14 days of adult release was recorded. Number of leaves with damage symptoms along with total leaves per plant was recorded after seven, 14 and 21 days of adult release and percent leaves with feeding symptoms was calculated.

(a) (b)

Figure 1. (a) Multiple-choice test inside the screen house (b) No-choice test with leaves in the plastic boxes.

No-choice test

No-choice test was conducted to determine host specificity of *Z. bicolorata* with 11 plant species of Asteraceae family and one plant species of Oleaceae family (Table 1). The test was conducted in laboratory of National Entomology Research Center during March- September, 2017 with three replications. The nursery of test plant species were maintained in the screen houses for regular supply of leaves. Insect rearing boxes with dimensions of 18.7 cm X 12.6 cm X 7.8 cm length, breadth and height (600 ml volume) were used for no-choice host specificity tests. Leaves of the test plant species were kept inside rearing boxes and two pairs of one week old adult *Z. bicolorata* were released. The petioles of leaves were covered with moistened cotton and wrapped with aluminum foil in order to prevent early senescence of leaves. The boxes were covered with lid having provision of ventilation and kept at 27±2ºC temperature in laboratory conditions (Figure 1b). Daily observation of adult feeding and oviposition was recorded. Deaths of adult insects recorded and mean adult longevity was

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calculated for each plant species. Old leaves were daily replaced with fresh ones after recording observation.

The second experiment on no-choice host-specificity test was conducted with the larvae of *Z. bicolorata*. Similar methodology was followed as in no-choice experiment with adult beetles but 10 third instar larvae were used instead of the adult *Z. bicolorata*. Daily observation on larval feeding was recorded. The larval and pupal periods were recorded for each plant species. The duration of larval survival was recorded on plant species, in which pupation did not occur. The adult emergences were also observed.

Statistical analysis

Data obtained from observations were entered into Microsoft Excel spreadsheets. Statistical analysis was carried with Genstat Discovery Edition 4. Data on number of adult beetles, eggs and larvae from multiple-choice test were log transformed and ANOVA (Analysis of Variance) was performed. Percent leaves with damage symptoms after seven and 14 days in multiple-choice test were square root transformed and analyzed. Data on percent leaves with damage symptoms after 14 days in multiple-choice test was analyzed (ANOVA) without transformation as percent data lying within 30-70% does not require transformation (Gomez and Gomez, 1984). Means were calculated for data on adult longevity and larval period/ survival duration obtained from the no-choice host-specificity tests.

RESULTS

Multiple-choice test

Average number of adult *Z. bicolorata* on plant species of multiple-choice test after different periods of insect release is given in Table 1. One week old adult beetles of *Z. bicolorata* were found moving to all plant species except to *D. pinnata*, *G. abyssinica* and *P. frutescence* after four hours. The highest number of adults was attracted to *P. hysterophorus* (9.00) followed by *B. pilosa* (1.00) and *L. sativa* (1.00). Whereas, *A. houstoniamum*, *C. indicum, H. annuus, X. strumarium* and *Z. elegans* recorded 0.67 adult beetles after four hours of insect release. One day after the release of adults, *A. houstoniamum, B. pilosa, C. indicum* and *X. strumarium* recorded 0.33 number of *Z. bicolorata*. *H. annuus* recorded 0.67 adult, and *D. pinnata* and *L. sativa* recorded 1.00 adult beetles. The adult insect population increased in *P. hysterophorus* (30.33) after 24 hours. No adult *Z. biclorata* was found on all plant species except *P. hysterophorus* after two days and onwards. Adult beetles on *P. hysterophorus* increased to 36.67 and 41.33 after two and three days, respectively. Thereafter, beetles on *P. hysterophorus* also decreased. The number of adult beetles on *P. hysterophorus* was recorded 34.00, 31.67 and 24.00 after seven, 14 and 21 days of insect release, respectively. The adults of *Z. bicolorata* initially moved to different plant species in multiple-choice test and shifted to *P. hysterophorus* over times.

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Table 2. Average number of adult *Z. bicolorata* settled on different plant species in multiple-choice test in screenhouse

SN	Plant species	Number of adults after different periods						
		4 hours	1 day	2 days	3 days	7 days	14 days	21 days
1	*Agerataum houstoniamum* Mill.	0.67(0.20)*	0.33(0.10)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
2	*Bidens pilosa* L.	1.00(0.30)	0.33(0.10)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
3	*Chrysanthemum indicum* L.	0.67(0.20)	0.33(0.10)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
4	*Dahlia pinnata* Cav	0.00(0.00)	1.00(0.26)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
5	*Guizotia abyssinica* L.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
6	*Helianthus annuus* L.	1.67(0.42)	0.67(0.16)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
7	*Lactuca sativa* L.	1.00(0.30)	1.00(0.30)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
8	*Parthenium hysterophorus* L.	9.00(1.00)	30.33(1.49)	36.67(1.57)	41.33(1.62)	34.00(1.54)	31.67(1.51)	24.00(1.39)
9	*Perilla frutescence* L.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
10	*Xanthium strumarium* L.	0.67(0.20)	0.33(0.10)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
11	*Zinnia elegans* Jacq.	0.67(0.20)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
P value		<.001	<.001	<.001	<.001	<.001	<.001	<.001
L.S.D.		0.194	0.273	0.025	0.035	0.027	0.017	0.040
CV (%)		44.3	67.6	10.4	14.1	11.3	7.4	18.6

*Value within parentheses are log transformed.

Average number of *Z. bicolorata* eggs on plant species of multiple-choice test is presented in Table 3. There was no oviposition on all plant species in multiple-choice test except *P. hysterophorus*. Eggs on *P. hysterophorus* plants were counted after two, four, seven and 14 days of insect release. Eggs were not counted after 21 days as most of the leaves were damaged by adult and larval feeding of *Z. bicolorata*. Eggs per plant on *P. hysterophorus* was found increased after two days of adult release and onwards. The eggs laid on *P. hysterophorus* plants were 5.33, 20.00, 176.30 and 256.30 per plant after two, four, seven and 14 days, respectively after the release of adult beetles. Thus, none of the plant species except *P. hysterophorus* was selected by the adult *Z. bicolorata* for ovipostion in multiple-choice test.

Average number of *Z. bicolorata* larvae on different plant species in multiple-choice test is given in Table 4. Larvae were not found on all the plant species in multiple-choice test except *P. hysterophorus* and larval feeding was also not seen. The number of larvae on *P. hysterophorus* was counted 30.33 per plant 7 days after release of the adult *Z. bicolorata*. The larval population increased during the second week after release and reached 78.83 larvae per plant after 14 days. Thus, it was confirmed that except *P. hysterophorus*, all the tested plant species were not suitable for larval development of *Z. biclorata* in multiple-choice test.

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Table 3. Average number of *Z. bicolorata* eggs on different plant species in multiple-choice test in screenhouse

SN	Plant species	Egg per plant after different periods			
		2 days	4 days	7 days	14 days
1	*Agerataum houstoniamum* Mill.	0.00(0.00)*	0.00(0.00)	0.00(0.00)	0.00(0.00)
2	*Bidens pilosa* L.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
3	*Chrysanthemum indicum* L.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
4	*Dahlia pinnata* Cav	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
5	*Guizotia abyssinica* L.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
6	*Helianthus annuus* L.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
7	*Lactuca sativa* L.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
8	*Parthenium hysterophorus* L.	5.33(0.79)	20.00(1.32)	176.30(2.25)	256.30(2.41)
9	*Perilla frutescence* L.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
10	*Xanthium strumarium* L.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
11	*Zinnia elegans* Jacq.	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
P value		<.001	<.001	<.001	<.001
L.S.D.		0.053	0.027	0.024	0.014
CV (%)		43.0	13.4	7.0	3.8

**Value within parentheses are log transformed.*

Percent of leaves with feeding symptoms in multiple-choice test after different periods of insect release is shown in Table 5. Feeding symptoms was observed only on *P. hystrophorus* in multiple-choice test. Both adults and larvae of *Z. bicolorata* avoided feeding on the other plant species. 21.26% leaves of *P. hysteroporus* were found with minor feeding symptoms 7 days after release of the insect. The percent leaves with feeding symptoms increased to 66.09% after 14 days. Nearly all leaves (98.52%) of *P. hysterphorus* were found with heavy feeding symptoms after 21 days of the adult *Z. bicolorata* release. The feeding symptoms were not prominent until second week as larval population was low and most of the larvae were in early instar, and symptoms were mainly due to adult feeding. The second weeks onward, their feeding symptoms on *P. hystrophorus* leaves were prominent as late instar larval population was high.

Table 4. Average number of *Z. bicolorata* larvae on different plant species in multiple-choice test in screenhouse

SN	Plant species	Number of larvae per plant after different periods	
		7 days	14 days
1	*Agerataum houstoniamum* Mill.	0.00(0.00)*	0.00(0.00)
2	*Bidens pilosa* L.	0.00(0.00)	0.00(0.00)
3	*Chrysanthemum indicum* L.	0.00(0.00)	0.00(0.00)
4	*Dahlia pinnata* Cav	0.00(0.00)	0.00(0.00)
5	*Guizotia abyssinica* L.	0.00(0.00)	0.00(0.00)
6	*Helianthus annuus* L.	0.00(0.00)	0.00(0.00)
7	*Lactuca sativa* L.	0.00(0.00)	0.00(0.00)
8	*Parthenium hysterophorus* L.	30.33(1.49)	78.33(1.90)
9	*Perilla frutescence* L.	0.00(0.00)	0.00(0.00)
10	*Xanthium strumarium* L.	0.00(0.00)	0.00(0.00)
11	*Zinnia elegans* Jacq.	0.00(0.00)	0.00(0.00)
P value		<.001	<.001
L.S.D.		0.039	0.027
CV (%)		17.0	9.1

**Value within parentheses are log transformed.*

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Table 5. Percent of leaves with feeding symptoms in multiple-choice test in screenhouse

SN	Plant species	Percent of leaves with feeding symptoms after different periods		
		7 days	14 days	21 days
1	*Agerataum houstoniamum* Mill.	0.00(0.71)*	0.00	0.00(0.71)
2	*Bidens pilosa* L.	0.00(0.71)	0.00	0.00(0.71)
3	*Chrysanthemum indicum* L.	0.00(0.71)	0.00	0.00(0.71)
4	*Dahlia pinnata* Cav	0.00(0.71)	0.00	0.00(0.71)
5	*Guizotia abyssinica* L.	0.00(0.71)	0.00	0.00(0.71)
6	*Helianthus annuus* L.	0.00(0.71)	0.00	0.00(0.71)
7	*Lactuca sativa* L.	0.00(0.71)	0.00	0.00(0.71)
8	*Parthenium hysterophorus* L.	21.26(4.66)	66.09	98.52(9.95)
9	*Perilla frutescence* L.	0.00(0.71)	0.00	0.00(0.71)
10	*Xanthium strumarium* L.	0.00(0.71)	0.00	0.00(0.71)
11	*Zinnia elegans* Jacq.	0.00(0.71)	0.00	0.00(0.71)
P value		<.001	<.001	<.001
L.S.D.		0.104	2.317	0.015
CV (%)		5.7	22.6	0.6

**Value within parentheses are square root transformed.*

No-choice test

No-choice tests of adult and larval stages of *Z. bicolorata* were conducted to determine host specificity on 12 different plant species in laboratory. Feeding symptoms, oviposition and longevity of adult *Z. bicolorata* on plant species in no-choice test is presented in Table 6. Feeding symptoms were not observed on all plant species except *H. annuus*, *X. strumarium* and *P. hysterophorus*. Feeding symptoms on *X. strumarium* and *P. hysterophorus* were observed from the second day of insect release. However, feeding on *H. annuus* initiated from the fifth day onward. Adult beetle starvation for five days enforced them to feed on *Helianthus annus* leaves in case of adult *Z. bicolorata* in no-choice test. Feeding on *H. annuus*, *X. strumarium* and *P. hysterophorus* was continuous throughout the adult life once initiated. As ovipostion is concerned, adults of *Z. bicolorta* laid eggs on leaves of *H. annus* and *P. hysterophorus.* No ovipositon was recorded in remaining all plant species including *X. strumarium*, though adults fed on its leaves. The oviposition on *P. hysterophorus* leaves was continuous throughout adult life. Eggs were laid on *H. annus* leaves only on the second and third day, then after no eggs were observed.

Table 6. Feeding symptoms, oviposition and longevity of the adult *Z. bicolorata* on plant species in no-choice host- specificity test in laboratory

SN	Plant species	Adult feeding symptoms	Oviposition	Adult longevity (days)*
1	*Agerataum houstoniamum* Mill.	No	No	13.17±2.62
2	*Bidens pilosa* L.	No	No	13.50±0.00
3	*Chrysanthemum indicum* L.	No	No	15.33±0.44
4	*Dahlia pinnata* Cav	No	No	8.17±0.44
5	*Guizotia abyssinica* L.	No	No	6.33±0.17
6	*Helianthus annuus* L.	Yes	Yes	19.00±1.00
7	*Lactuca sativa* L.	No	No	17.83±2.62
8	*Parthenium hysterophorus* L.	Yes	Yes	83.33±2.09
9	*Perilla frutescence* L.	No	No	13.50±2.52
10	*Xanthium strumarium* L.	Yes	No	29.33±1.88
11	*Zinnia elegans* Jacq.	No	No	12.50±0.00
12	*Jasminum officinale* L.	No	No	8.67±1.92

**Values presented as Mean±SEM (Standard Error of Mean)*

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Adult longevity on different plant species without feeding was shorter compared to adult longevity on plant species consumed by beetles. The adult longevity ranged 6.33 to 17.83 days in plant species without feeding symptoms, while it was 19.00 days in *H. annus* and 29.33 days in *X. strumarium*. The longest adult longevity was recorded on host plant *P. hysterophorus* (83.33 days).

Table 7. Larval feeding symptoms, pupation, adult emergence and larval period/survival duration of *Z. bicolorata* on plant species in no-choice test in laboratory

SN	Plant species	Larval feeding	Pupation	Adult emergence	Larval period/survival duration (days)*
1	*Agerataum houstoniamum* Mill.	No	No	No	5.00±0.00
2	*Bidens pilosa* L.	No	No	No	3.67±0.33
3	*Chrysanthemum indicum* L.	No	No	No	4.67±0.33
4	*Dahlia pinnata* Cav	No	No	No	3.33±0.33
5	*Guizotia abyssinica* L.	No	No	No	3.33±0.33
6	*Helianthus annuus* L.	Yes	No	No	10.33±2.96
7	*Lactuca sativa* L.	No	No	No	5.00±0.00
8	*Parthenium hysterophorus* L.	Yes	Yes	Yes	7.00±0.58
9	*Perilla frutescence* L.	No	No	No	3.33±0.33
10	*Xanthium strumarium* L.	Yes	Yes	Yes	10.33±0.33
11	*Zinnia elegans* Jacq.	No	No	No	3.00±0.00
12	*Jasminum officinale* L.	No	No	No	2.66±0.33

**Values presented as Mean±SEM (Standard Error of Mean)*

Data recorded from no-choice test with larvae of *Z. bicolorata* is presented in Table 7. Larval feeding was observed only on three plant species, *H. annus*, *X. strumarium* and *P. hysterophorus*. The larvae fed on *H. annus* could not pupate though survived for 10.33 days; the larvae fed on *X. strumarium* pupated after 10.33 days, while larvae pupated in 7.00 days on *P. hysterophorus.* Adult emergence occurred in both *P. hysterophorus* and *X. strumarium.* Immature stage development occurred on *X. strumarium* though larval period was somewhat longer than the host plant *P. hysterophorus*.

DISCUSSION

Under multiple-choice test conditions, one week old adult *Z. bicolorata* fed only on *P. hysterophorus.* The insect observed on other plant species apart from *P. hysterophours* till 24 hour aggregated to *P. hysterophorus* after 48 hours of insect release. Host plant finding and acceptance in the field involves different behaviors mediated through sensory cues (Bernays and Chapman, 1994). Visual and olfactory cues are mostly important during pre-alighting stage and is common to wide range of plant species (e.g. green color, non-specific plant odor) in host finding process (Maharosy,1998). Insect responds to physical and chemical stimuli of the plant after making contact with a plant. Parthenin, a sesqueterpene lactone is responsible to induce feeding of *Z. bicolorata* and which is specific to *P. hysterophorus* (Jayant *et al.*, 1993). Considering facts, *Z. bicolorata* moved to other plant species because of common visual stimuli and later shifted to *P. hysterophorus* as physical and chemical cues do not fulfill their requirement. McClay (1980) also reported adults of *Z. bicolorata* previously fed on *P. hysterophorus* did not feed on other plant species even in confinement.

Adults of *Z. bicolorata* oviposited only on *P. hysterophorus* among plant species tested and eggs were not recorded from the rest of the plant species in multiple-choice test. Similarly,

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larvae were recorded only from *P. hysterophorus* and not on other plant species. Both the adult and the larval feeding were confined to *P. hysterophorus* among the tested plant species in present study under multiple-choice test. Similar finding was reported by Jayanth and Nagarkatti (1987) while investigating host specificity and damage potential of *Z. bicolorata* on 40 plant species, in which adult feeding, oviposition and larval feeding were not observed on 37 plant species. They found adult and larval feeding only on *P. hysterophorus* among tested plant species and slight adult feeding was observed on *Jasminum gandiflorum* L. and *G. abyssinica* in multiple-choice test. Mersie *et al*. (2018) studied the host range of *Z. bicolorata* and evaluated against 29 non-target plant species in Ethiopia and found that *Z. bicolorata* did not oviposit and feed on non-target plant species in multiple-choice test. Similarly, Malkapure *et al*. (2012) evaluated feeding preference of *Z. bicolorata* on *P. hysterophorus*, *Carthamus tinctorius* L., *H. annus*, *C. indicum*, *Tagetes erecta* L., *G. abyssinica* and *X. strumarium*, and reported only *P. hysterophorus* preferred by *Z. bicolorata* as both the larvae and the adult fed on it.

In no-choice tests, adults as well as larvae of *Z. bicolorata* were found to feed on *H. annuus* and *X. strumarium* apart from *P. hsyterophorus* in the present study. However, the adult longevity of beetles fed on *H. annus* and *X. strumarium* was found quite shorter compared to adults fed on *P. hysterophorus*. McClay (1980) tested host specificity of *Z. bicolorata* previously described as *Zygogramma sp*. near Malvae Stal. for the first time on various plant species and found newly emerged adults slightly feeding on *H. annuus* but larvae were not found feeding on it. *Z. bicolorata* was found to feed on the tender leaves of sunflower (*H. annuus*) at few place of Karnataka state in India (Jayant *et al.*, 1993). Bhute (2012) recorded adult beetles of *Z. bicolorata* feeding on *X. strumarium* after defoliation of *P. hsyterophorus* in the experimental plots.

Adult beetles on *H. annus* stated feeding only five days after release probably due to starvation. Similar findings about feeding on non-host plant species due to starvation had been reported by Wither (1999). He studied feeding behavior of *Z. bicolorata* on high ranked host *P. hysterophorus* and low ranked host *Xanthium occidentale* L.. Adult *Z. bicolorata* beetles that had just fed, or were one inter-meal interval after feeding, or were deprived for six days after feeding, were used. Result showed that just-fed beetles were generally unresponsive to either host plant and did not feed. Beetles held for one inter-meal interval showed more feeding responses towards the higher ranked plant. Six days food deprived beetles showed less difference in feeding responses between the two plants, but still discrimination existed between the two plants.

Adult *Z. bicolorata* laid few eggs on *H. annus* for the first two days and stopped thereafter in no-choice experiment. Ovipostion did not occur on *X. strumarium* though adult fed on it. McConnachie (2015) conducted multiple-choice and no-choice test for *Z. bicolorata* involving 48 plant species. He found that adults selected *H. annuus* and *X. strumarium* for feeding and oviposition though *P. hsyterophorus* was significantly preferred. Adults of *Z. bicolorata* fed on sunflower leaves for one month were incapable of laying eggs; they resumed oviposition only after feeding on *P. hsyterophorus* for 7-10 days (Jayant *et al.*, 1993). The larvae fed on *X. strumarium* pupated and adult emerged from it but larvae could not pupate when fed on *H. annuus* in the present study. McConnachie (2015) reported larval development significantly higher in *P. hsyterophorus*, and partial and reduced in *H. annuus* and *X. strumarium*. Bhute

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(2012) reported that *Z. bicolorata* could survive on *X. strumarium* in absence of *P. hsyterophorus*.

CONCLUSION

Major findings from multiple-choice and no-choice host specificity tests of *Z. bicolorata* on 12 taxonomically related plant species are summarized as follows:

- Only *P. hysterophorus* was preferred host of *Z. bicolorata* among host-specificity tested plant species. Both the larvae and the adults fed on it, and oviposition, pupation and adult emergence occurred successfully completing life cycle on *P. hysterophorus*.
- Larvae of *Z. bicolorata* consumed *H. anuus* in no-choice test but larvae did not complete its development. Adults consumed *H. annus* after starvation for 5 days in no-choice test. Adult longevity was shorter and adults did not oviposit after feeding on *H. annus*.
- *X. strumarium* was readily consumed by both the adults and the larvae of *Z. bicolorata* in no-choice test. Though the larva and the pupa completed its development on *X. strumarium*, but adult did not oviposit. Adult longevity was significantly reduced after feeding on *X. strumarium*.

Thus, we can conclude that *Z. bicolorata* is *P. hysterophorus* specific bio-control agent and do not pose threat to taxonomically related non-target cultivated and wild plant species. This bio-control agent can be mass multiplied and augmentative release can be done against *P. hysterophours* in Nepal. Host-specific nature of *Z. bicolorata* will help the insect to establish, spread and self-sustain on *P. hysterophorus*. This weed controlling insect could be used in future for sustainable and environment friendly management of invasive and health hazardous *P. hysterophorus* in Nepal.

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Authors' contributions

Ajaya Shree Ratna Bajracharya conducted and wrote this paper. R. B. Thapa, G. B. KC, S. B, Pradhan and J. D. Ranjit supervised research and revised the paper.

Conflict of interest

This manuscript is original and free from any plagiarism, and has not been published before and is not currently being considered for publication elsewhere. There is no conflict of interest associated with this publication.

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