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Biocontrol of Parthenium Hysterophorus using *Zygogramma Bicolorata*: Implications for Maize Grain Yield

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ABSTRACT

Biological control is a cost-effective and sustainable method many countries use to control invasive alien plant species. This study was conducted to establish the effectiveness of Z. bicolorata in controlling the noxious weed crop Parthenium hysterophorus and the associated benefits in enhancing maize yield performance under screen house conditions. The R-studio software was used to analyze data on plant height at flowering, number of leaves, leaf length, plant height at maturity, number of plants harvested, ear length, ear diameter, number of kernels per ear, number of kernels per row, plant biomass and grain yield. Maize yields were considerably higher (1800 g) in plots without parthenium than those with parthenium and no beetles (322 g). In plots with parthenium and the biocontrol agent maize yields were 1617 g, evidence that the agent effectively reduces the competitive ability of parthenium weed under these conditions. In addition, maize plants in plots with no parthenium weed and those with parthenium weed and Z. bicolorata were significantly taller, had longer leaves, had more kernel rows and kernels per ear and had higher plant biomass compared to those maize plants growing together with parthenium in the absence of the biocontrol agent. Furthermore, the study found that defoliation of parthenium weed by Z. bicolorata contributed to a significant decrease in plant height, root length, number of flowers per plant and plant biomass of this invasive plant. Zygogramma bicolorata can significantly suppress the growth of parthenium weed in maize cultivation plots and thus enhance the growth and yield of maize.

Keywords: Bioagent, Grain yield**,** Maize, *Parthenium hysterophorus*, *Zygogramma bicolorata*.

1.0 INTRODUCTION

Weeds are plants in the wrong place [1]. In the tropics, weeds cause more crop losses, and farmers spend more of their time weeding crops than in any other part of the world. Estimated yield losses caused by weeds in Africa amount to 16% compared to only 7% in Europe [2]. One known case is the *Parthenium hysterophorus*, which is now considered a damaging weed on crops [3]. It is considered a noxious weed because of its allelopathic effect, its strong competitiveness for soil moisture and nutrients [[4]. Crop losses are caused primarily by allelopathic effects, more so than its ability to compete for nutrients and moisture and these losses are often proportionally higher than expected from a similar crop weed [5]. Another mechanism by which parthenium impacts crop productivity is through its ability to cover crops in pollen, which prevents seed set, reducing yields of up to 40% [6]. In Ethiopia, the yield of sorghum was severely reduced to 97 % at the lowland site, as reported by Tamado and Milberg [7], while in India, yields were reduced from 6.5 to 4.3 t ha^{-1} [8]. *Parthenium hysterophorus* also reduced the growth of maize (*Zea mays*) especially plant height, dry biomass, corn weight, and corn length and grain weight per corn by 21.1%, 42.3%, 50.9%, 51.1% and 52.2%, respectively [[9]. Parthenium weed was reported to reduce forage production in grasslands by up to 90% in India [[7]. However substantial yield loss in sunflower and sorghum in central Queensland, Australia was also reported [9]. In Kenya, it is reported to be one of the important weeds in coffee [10]. But also, parthenium weed seeds are also a contaminant of grain, pasture seed and forage, resulting in restricted sale and movement of these produces

[11]. In the Caribbean, where crop losses due to weeds average about 20% while parthenium contributed as the top weed in the losses largely because of its tolerance to the widely used herbicide paraquat [12]. In Tanzania, a study was done to determine the effects of *Parthenium hysterophorus* on other native herbaceous plant species in rangeland, and the result showed that the parthenium weed had a substantial threat to other herbaceous plants which are the source of food for both wildlife and livestock [13].

To reduce these impacts, it is imperative that control strategies be developed and implemented. Management interventions should focus on prevention, eradication of small isolated populations, and the use of biological control for widespread invasions [14]. Biological control is particularly attractive as a long-term strategy, as it is inexpensive, self-sustaining and permanent [15]. Several biocontrol agents including *Zygogramma bicolorata* have been released around the world for the control of parthenium weed [16]. In India, *Z. bicolorata* caused widespread defoliation of parthenium weed leading to biodiversity recovery [17]. Reports from Australia suggest that the beetle caused a significant decline in parthenium weed populations [18]. However, no studies have evaluated the benefits of parthenium weed defoliation by *Z. bicolorata* on maize yields in Tanzania. As such the objective of this study was (i) to assess the effectiveness of *Z. bicolorata* as a biological control agent of parthenium weed and (ii) to evaluate its effect on improving maize yields after suppressing parthenium weed under screen house conditions.

2.0 MATERIALS AND METHODS

2.1 Experimental details

2.1.1 Experimental design and layout

Trials were undertaken in a large screen house at the Plant Protection Division, Tanzania Plant Health and Pesticides Authority (TPHPA) (Figure 1). Twelve 3x2 m semi-enclosed cages were constructed in the screen house allowing for four replicates of each of three treatments. The three treatments consisted of plantings of maize alone, parthenium weed and maize, and parthenium weed and maize together with *Z. bicolorata*. Ten pairs of *Z. bicolorata* adults were hand-placed in the semi-enclosed plots with maize and parthenium, with no beetles in the other treatments

Figure 1: Large screen house at the Tanzania Plant Health and Pesticides Authority (TPHPA) in which the trials were undertaken.

2.1.2 Land preparation, sowing and management practices

The land was prepared by hand hoe. The stubbles and weeds were removed from the experimental area and the soil was brought to fine tilth. Plots were irrigated and left to drain for one day. The following day fifty parthenium seedlings were planted in each plot one week before the planting of SC 403 hybrid maize seeds. Three maize seeds were sown in each ridge between rows of parthenium seedlings. Maize seeds were planted at least 60 cm from each

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other within a ridge with a distance of 75 cm between ridges at a depth of 5 cm. A 1.0 m pathway was maintained around the entire experimental area. The selected maize hybrid was due to characteristics such as very early maturity and mottle viruses' tolerance. The maturity period is relatively short and is about 90 days. Has flinty ears and excellent yield stability over a range of environments (Figure 2).

Dead parthenium seedlings were replaced one week after initial planting while ungerminated maize seeds were replaced a week after sowing. Of the three maize seedlings in each ridge, one was removed after 15 days in order to maintain two plants which can grow properly. 250 g of urea fertilizer was applied in each plot three weeks after maize sowing. Plants were once watered when required (Figure 2).

Figure 2: Treatment plots showing parthenium seedlings (top left); plot with large parthenium plants and maize (top right); plot with maize only (bottom left).

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2.2 Data collection

Each plot had five rows of planted maize plants and parthenium weeds. The collection of maize data was done from ten (10) maize plants in the two middle rows. The total number of leaves of 10 flowering maize plants was recorded together with the length of randomly selected leaves per plant. Maize plant height at maturity (cm) was obtained by taking an average plant height of 10 maize plants in the two middles. Twenty maize plants were harvested and recorded while ear length and diameter (cm) were obtained by measuring a maize ear from peduncle to end of an ear and the diameter was measured by breaking a maize ear at the middle and measuring a length at the middle using a ruler from one kernel to other kernels of the opposite side. Number of kernel rows/ear and the total number of kernels/rows were obtained by counting the number of kernels per ear for 10 randomly selected maize ears. Plant biomass was obtained by uprooting a maize plant, washing out all soil particles then drying it in an oven at 55^0C for 72 h and dry weight was recorded from twenty maize plants obtained from two middle rows. Finally, 100 maize seeds from each plot were harvested and weighed to obtain maize grain yield. Plant height, root length, number of flowers per plant and plant biomass of each parthenium plant in each plot were recorded at the end of the experiment.

2.3 Data analysis

All plant variables were entered into an Excel spreadsheet for preliminary analysis. Thereafter, all data were imported into R-studio software to check for normality tests. The normality test of data was done by using Shapiro-Wilk's test. After the test, all data were normally distributed.

To determine the effect of *Z. bicolorata* against *P. hysterophorus* on maize yields, maize data on plant height at flowering, number of leaves, leaf length, plant height at maturity, number of plants harvested, ear length, ear diameter, number of kernels per ear, number of kernels per row, plant biomass and grain yield were analysed by One-way analysis of variance whereby the statistical significant difference was measured at $p<0.05$. Differences between the means were compared using the Tukey post hoc test. On the other hand plant height, root length, defoliation percentage by *Z. bicolorata*, total plant biomass and number of flowers produced per plant to prove the effectiveness of *Z. bicolorata* against parthenium weed plant in plots with and without *Z. bicolorata* were compared by using student *t*-test.

3.0 RESULTS

3.1 Impact of *Zygogramma bicolorata* on *Parthenium hysterophorus*

Z. bicolorata significantly negatively affected plant height, flower production, root length, plant biomass and defoliation (in all cases $P < 0.05$). There was a large difference in mean plant height in plots present with *Z. bicolorata* compared to plots absent with *Z. bicolorata* beetles (Table 1). Moreover, there were a statistical significant differences in plant biomass (t = -7.08, $P < 0.05$) in plots present with *Z. bicolorata* compared to plots that had no *Z. bicolorata* (Table 1). But also the average root lengths in plots present with *Z. bicolorata* were statistically significant, unlike plots with no *Z. bicolorata* beetles ($P< 0.001$). Moreover, there was a significant difference in percentage of the defoliation between plots with *Z. bicolorata* compared to plots with no *Z. bicolorata* beetles (t = 16.44, $P < 0.001$) (Table 1).

Table 1: Impact of *Zygogrammabicolorata* on *Parthenium hysterophorus* after 90 days in plots with (present) and without (absent) this classical biological control agent

Parameter	Mean ± S.E	ean ± S.E	*t*-value	*P*-value
	Present	**Absent**		
Plant height (cm)	47.175±5.9137	141.0±9.210	-10.22	P<0.001
Flower production/plant	1.75±6.88	100.1±26.543	-7.31	P<0.001
Root length (cm)	8.04±1.12475	15.535±1.207	-6.47	P<0.001
Plant biomass (g/plant)	6.75±1.594	35.7±4.895	-7.08	P<0.001
Defoliation % by *Z. bicolorata*	88%±8.899	8% ±2.844	16.44	P<0.001

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3.2 Suppression of *P. hysterophorus* by *Z. bicolorata* and its effects on maize grain yield

From the result, maize yields were higher (1671 g) in plots where *Z. bicolorata* was controlling parthenium than in those where the biocontrol agent was absent (322 g). In plots without any parthenium, maize yields were 1800 g (Table 2). On the other hand, there were no significant differences in plant height at maturity in all treatments (in all cases $P > 0.05$). But also, the ear diameter and plant biomass also showed no significant difference in plots where parthenium was controlled by *Z. bicolorata* and in plots where the biocontrol agent was absent (Table 2). In plots without any parthenium weed but with maize and beetles the ear diameter and plant biomass showed significant differences indicating a positive contribution to yield gain (Table 2). Furthermore, on all other remaining variables, significant difference was shown at which the $P < 0.005$ (Table 2).

Table 2: The mean of measured variables of the maize plant that contributed to maize grain yield between different treatments

Variables		**Mean**		***F*-value**	***P*-value**
Factors	**Parthenium with maize and beetles**	**Parthenium with maize and no beetles**	**Maize with beetles and no parthenium**		
Plant height at flowering (cm)	49.09 b	27.06 c	67.74 a	88.98	0.00
Number of leaves	10.05 b	8.9 c	10.83 a	21.6	0.00
Leaf length (cm)	50.744 b	33.87 c	77.38 a	58.51	0.00
Plant height at maturity (cm)	1.1825a	0.95 a	3.69 a	0.66	0.536
Ear length (m)	0.1 b	0.0602 c	0.1391a	16.83	0.001
Ear diameter (m)	0.0145 a	0.00475 a	0.03385 b	10.56	0.33025
Number of kernel rows per ear	11.85 b	3.15 c	13.25 a	324.61	0
Number of kernel per row	12.575 b	2.425 c	14.55 a	23.95	0
Plant biomass (g)	41.75 b	19.25 b	102 a	23.26	0.22592
Grain yield (g)	1617 a	322b	1865 a	13.97	0.002

*****Means that do not share a letter within a row are significantly different.

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Figure 3: Parthenium plants defoliated by *Zygogramma bicolorata* after 90 days

4.0 DISCUSSION

4.1. Impact of *Zygogramma bicolorata* on *Parthenium hysterophorus*

Based on our findings both *Zygogramma bicolorata* larvaes and adults contributed to the almost total defoliation of parthenium plants which impacted negatively on plant height, flower production, root length and plant biomass hence affecting plant performance on growth, reproduction and fitness (Table 1). These defoliation activities could be attributed to the feeding behaviour of both larvae and adults, which tend to congregate and feed on tender leaves, terminal and axillary buds and thereby leading to stunted growth.

The results obtained are in contrast with the results given by Shushilkumar[[16] that even after the release and establishment of *Z. bicolorata* on parthenium-infested sites, still a desirable control of parthenium weed was not achieved and this was facilitated by slow population buildup of the *Z. bicolorata* beetles and intermittent emergence from diapause.

The present study shows that there was a big mean difference in plant height and biomass in plots having *Z. bicolorata* compared to plots that are absent with the presence of *Z. bicolorata.* Their differences could be due to the reasons that *P. hysterophorus* plants were severely damadeg by *Z. bicolorata*, which probably resulted into a decrease in height and above fresh biomass. But also, could be due to the reason that, when plants have few leaves, the supply of nectar is reduced, seed dispersion becomes poor, and hence biomass decreases in plants, since plants will have a harder time of surviving in light competition and become more prone to petal and sepal abscission. Our result correlates with the result found by (Shabir *et al*., 2016) that the plant height and dry biomass were significantly compromised after the introduction of *Z.bicolorata*. The results are also in congruence with the result reported by Dhileepan [19] that the reduction in height of the parthenium weed plant was significantly effective due to the initial release of Z. *bicolorata* at the rosette stage but also due to continuous feeding on the vegetative apical meristems.

The present study also reports that the root length was significantly reduced in plots with *Z. bicolorata* compared to plots without *Z. bicolorata*. Such influence of defoliation as a result of the reduction in root length might be due to high pressure of defoliation on the Parthenium weed plant which has resulted on the reallocation of resources from root to shoot regrowth hence the reduction in root biomass which likely reduces the root length. Similar results have been shown by Shushilkumar [16] that extended defoliation pressure by *Z. bicolorata* on parthenium weed results in

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the reallocation of resources from the root to shoot regeneration with a significant reduction in root biomass and flower production. On the other hand plots without *Z. bicolorata,* the root length was high, this is presumably due to incompetativeness for food and space between larvae and adults of *Z. bicolorata*. But also, favourable weather and soil conditions with high organic matter could have favoured a continuous growth and increase of the root length.

The reduction in flower production on plots with *Z. bicolorata* beetles was due to the early release of Z. *bicolorata* at the early stage of parthenium plant growth, whereby female adults targeted the flower buds for egg laying, and after hatching the neonate fed on the flowers and this prevented the production of parthenium seeds. But also, the reduction in flower production might have been due to synchrony between parthenium weed germination and the establishment of Z. *bicolorata* in cages as a result both larvae and adults damaged all the plant meristem. Similarly, Hasan *et al*., [20] reported that 98% of defoliation of parthenium plants does not produce flowers and seeds.

Moreover, the reduction in parthenium biomass was due to a large reduction in plant density since the *Z. bicolorata* population built up very well at the initial stage of the parthenium plant growth and thereby reduced seed production. Similar results were reported by Shushilkumar [16] that upon augmentative release of the Mexican beetles for three years, the beetles caused a reduction of the parthenium plant biomass which could probably be due to the decrease in plant density as a result of the reduction of seed production due to the beetle herbivory before flowering.

From the present study, results indicate that defoliation percentage by *Z. bicolorata* was highly significantly reduced by 88% in plots with *Z. bicolorata* compared to plots without *Z. bicolorata*. The defoliation impact might be due early population buildup of both larvae and adults of *Z. bicolorata* that likely caused excessive damage to the primary meristems, reduced flower production as a result of changing branching pattern and reduced plant height of parthenium weed plants. Similar results were reported in the study by Madrewar *et al*. [21] which reported that there was 80% reduction in Parthenium density in plots with *Zygograma bicolorata*.

The present results are in congruence with the result given by Dogra and Rashid [22] that a single adult *Z. bicolorata* per plant caused 85–100% defoliation within six to eight weeks, but this depended on the stage of the plant growth.

4.2 Suppression of *P. hysterophorus* by *Z. bicolorata* and its effects on maize grain yield

From the result, a greater yield of maize grain was observed in plots with maize only, no parthenium weeds but had *Z. bicolorata*. This might be due to that *Z. bicolorata* did not attack any maize plant and hence maize growth proceeded without any disturbance as a result of yield gain. Furthermore, the result show that plots that had parthenium weeds, and maize but were not controlled with *Z. bicolorata* had low maize grain yield compared to those with maize only. This might be due to resource competition such as soil nutrients, which could all be taken by parthenium weeds at their growth time, development of many branches that limit penetration of sunlight to reach nearby vegetation for our case maize crops. But also, the weed produces an enormous quantity of pollen (parthenin) which is carried and settles on vegetative and floral parts (on stigmatic surface) of a plant hence inhibiting fruit setting in crops hence low yield. A similar result has also been given by Tamado and Milberg [11] that inhibition of the activity of nitrogen-fixing and nitrifying bacteria has led to yield loss in tomato, maize, beans and brinjal. Nishanthan *et al*., [23] also reported that heavy accumulation of pollen of *P. hysterophorus* on the stigmatic surface of tomato has caused a 40% reduction in crop yield and that the weed may still exhibit an inhibitory influence on crops even when grown at a considerable distance.

5.0 CONCLUSION AND RECOMMENDATIONS

Conclusively the results showed that *Z.bicolorata* greatly affected all parthenium weeds variables. Its effectiveness was found to be more when *Z.bicolorata* beetles were released at the initial stage of parthenium growth and before flowering. Moreover, the study found out that as the number of *Z. bicolorata* became high there was an increasing damage to parthenium plant meristems thus significantly minimizing the soil seed bank therefore resulting in the reduction of parthenium weed density thus permitting maize crops to grow. Generally, the present study indicates that the use of *Z.bicolorata* would help suppress the growth and development of the parthenium weed and enhance the yield of maize.

Moreover, the study recommends that, for effective biological control of parthenium weed, there should be a massive time-after-time release of *Z. bicolorata* adults at the initial stages of parthenium plant growth and maintained for a long period of time. The study recommends for timely adult release simply because adults are responsible for the population build-up and propagation of the new colony for better performance and effective management of parthenium.

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Nevertheless, the present study was undertaken in a limited area (on a semi enclosed cages) but if the future studies can be made on a large scale involving an integrated approach such as the development of effective environmentally-friendly new herbicides such as the use of allelochemicals of allelopathic trees, sowing of healthy pasture seed, the introduction and development of other biological control agents like the stem-boring weevil *Listronotus setosipennis*, the seed-feeding weevil *Smicronyx lutulentus* and adapting existing cultural practices such as uprooting and burning the weed, this will help to eradicate this aggressive weed as it has already spread very quickly in Tanzania.

CRediT authorship contribution statement

Joyce Christopher: Conceptualization, methodology, data collection, software, formal analysis, initial manuscript preparation and project administration; Gerubin L. Msaki: Conceptualization formal analysis, initial manuscript preparation, Review and Editing.

Declaration of competing interest

The authors declare no conflict of interest, as they are not affiliated with any organization or entity with any financial or non-financial interest in the subject matter discussed in this paper.

Data availability statement

All relevant data are included in the paper

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