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The first report of GPS-based mapping of Parthenium beetle (Zygogramma bicolorata Pallister) occurrence, distribution and its impact on Parthenium (*Parthenium hysterophorus* L.) in Northern Sri Lanka

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ABSTRACT

Purpose: *Parthenium hysterophorus L. is a globally recognized one of the most invasive noxious weeds. It has invaded more than 17 districts in Sri Lanka, and is listed under weed of national significance for eradication. Biological control using parthenium beetle Zygogramma bicolorata is one of the promising eco-friendly strategies for Parthenium management and is successful in many countries.*

Research Method: *The biennial survey was conducted to explore the potential biocontrol agent Z. bicolarata of the parthenium and its dispersal around Northern Sri Lanka. Preliminary survey data identified sampling sites that were chosen to draw the distribution and density maps of the Parthenium beetle (Z. bicolarata) using GPS tools. The field and greenhouse damage incidence of Z. bicolarata on Parthenium was calculated and subjected to ANOVA using SAS 9.1. Tukey's HSD multiple comparison tests to identify the best treatment combination at P <0.05.*

Findings: *The results exhibit that the Z. bicolarata distribution is confined to the Jaffna district in Northern Sri Lanka, and occurrence was the first time in Valikamam North in the year 2019/20. The highest density of the Z. bicolorata population was 35-62 per m^2 in the Valikamam North DS division in the years 2019/20 and 2020/21, respectively. The damage incidence varied from <10%-100%, but maximum field damage was 25->50% in Valikamam North whereas in the greenhouse 10 grubs inflicted 75-100% defoliation in 9±0.75 days.*

Originality/value: *This is the first report of the occurrence of Z. bicolorata and a detailed study of its distribution, and damage to Parthenium to decide the biological Parthenium control program.*

Keywords: *Biocontrol, GPS mapping, Occurrence, Parthenium beetle, Zygogramma bicolorata*

INTRODUCTION

The most noxious invasive and aggressive terrestrial weed Parthenium (*Parthenium hysterophorus* L.) originated in the Gulf of Mexico, and invaded more than 94 countries situated on five continents and numerous islands within a short period using its biological power (Adkins, *et al.*, 2018; Gnanavel and Natarajan, 2013). It is a resilient annual herb belonging to the family Asteraceae (Heliantheae) and adapted to survive a wide range of soil and climatic conditions (Evans 1997; Khaket *et al.*, 2012). Therefore, if Parthenium is accidentally or deliberately introduced in a particular locality,

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it can a pose constant threat and huge economic damage to agriculture, animal husbandry, and the eco-system (Gnanavel and Natarajan, 2013; Saini, *et al.*, 2014; Nishanthan *et al.*, 2013). Hence, stringent legislative and preventive measures are the best option to escape from the menace.

Invasion of the Parthenium was detected first time in Northern Sri Lanka (Vavuniya district) in 1999, and scientific investigations concluded that Parthenium was introduced from India through sheep brought by the Indian Peace Keeping Force (IPKF) for their consumption while entering into the country in 1987 (Jayasuriya, 1999; Jayasuriya, 2021; Marambe *et al.*, 2011). Since the incursion, Parthenium has spread to 17 districts of Sri Lanka and northern Sri Lanka has a high density of Parthenium population (Kishojini *et al.*, 2018). Therefore, the frequent survey of the occurrence and dispersal pattern of the Parthenium and the fauna associated with the weed using sophisticated digital methods is opened quickest pathway to monitor their dispersal for wider coverage to develop effective management strategies (Royimani *et al.*, 2019). GIS base tool kits using ArcGIS, remote sensing using MaxEnt (Maximum Entropy Algorithm), and random forest classification are such new methods (Kishojini *et al.*, 2018; Mushtaq *et al.*, 2021; Royimani *et al.*, 2019).

The climatic conditions of Sri Lanka, rapid infrastructure development, biological attributes of the Parthenium (short life cycle, an allelopathic potential, a C3/C4 photosynthetic mechanism, low nuclear "DNA content, phenotypic plasticity in its growth form, an efficient reproductive system including small seeds and mechanism for efficient spread), high tolerance to abiotic stresses and lack of natural control factors (pests and diseases), facilitated the quick and massive dispersal of this noxious weed, therefore, listed as a new invasive weed of national significance by the SLCARP and Department of Agriculture (DOA) (Jayasuriya, 2001; Shrestha *et al.*, 2019). Several management strategies such as quarantine, cultural, physical, and chemical have been proposed and implemented to eradicate the Parthenium weed from Sri Lanka. Parthenium control by manual and chemical weed control strategies is effective in agricultural fields, these methods are not cost-effective in pastures, vast natural areas, or wastelands. The chemical approach was a preferred means in the past three to four decades, but in Sri Lanka, many potential herbicides have been banned due to growing concern about chronic human health hazards (Marambe *et al.*, 2008; 2011). Therefore, the cost-effective, environmentally friendly, and ecologically viable method of controlling Parthenium weed is biological control (Shabbir *et al.*, 2016).

Several types of biological control agents (BCA) such as insect pests, and disease-causing agents of the Parthenium have been reported all around the world (Adkins *et al.*, 2018). Among BCA, Parthenium beetle (*Zygogramma bicolorata* Pallister) is most effectively being used to manage Parthenium in tropical countries (Dhileepan and Wilmot Senaratne 2009). Grub and adult *Z. bicolorata* beetles feed on leaves, meristem, and flower buds (Dhileepan 2009; Dhileepan and Wilmot Senaratne 2009). Defoliation decreased plant height and flower production even when there was no water stress, and the reductions were more pronounced when the plants were defoliated early in their growth cycle (Dhileepan *et al.*, 2000; Dhileepan 2003).

According to Jayasuriya (2021), the stem-galling moth *Epiblema strenuana* (Walker) and the summer rust *Puccinia xanthii* var. *parthenii-hysterophorae* (previously known as *P. melampodii*) were imported from Mexico to Sri Lanka in 2003. Stem-galling moth *E. strenuana* was field released in 2004 after completion of quarantine studies, but has not been observed so far in the dry-zone of Sri Lanka. Efforts made in 2003 to introduce the leaf-feeding beetle *Z. bicolorata* were not successful due to the failure to rear a culture under quarantine conditions (Dhileepan and Wilmot Senaratne 2009). Pakeerathan (2009) reported that the presence of *Z. bicolorata* in Northern Sri Lanka. Therefore, more field surveys are needed to determine the current status of BCA of Parthenium and its impact on Parthenium. Such scientific information is essential for planning and implementing the appropriate biocontrol strategies.

Therefore, an investigation was planned to draw a map of the distribution of *Z. bicolorata* using sophisticated GPS tools and to measure the impact of *Z. bicolorata* on Parthenium.

MATERIAL AND METHODS

As per the 2017 Parthenium distribution map, the biennial survey was started in 2017 to explore the potential biocontrol agents of the Parthenium and its dispersal in all the districts around Northern Sri Lanka such as *Jaffna*, *Kilinochchi*, *Mullaitivu*, *Vavuniya*, and *Mannar*. Preliminary survey data identified sampling sites were chosen to collect the data on the distribution and density maps of Parthenium beetle (*Z. bicolarata*), and damage incidence of *Z. bicolarata* on Parthenium.

Geographical Positioning System (GPS) Based Mapping of Parthenium Beetle

All the Parthenium-identified districts were classified into the Divisional Secretariat (DS) division (Kishojini *et al.*, 2018). The location of the occurrence of *Z. bicolarata* was accurately positioned and exact coordinate points indicating the distribution of *Z. bicolarata* were taken with the help of a GPS device randomly and opportunistic observation in the years 2019/2020 October-March and the same locations were sampled repeatedly in 2020/2021 April- March using tagged marks and GPS coordinates. Collected data were imported into ArcGIS_10.8 using the Add Data tool. The data points were mapped on the previously published background map to generate a desired new map to show the distribution of *P. hysterophorus* and *Z. bicolarata*. Editing tools of the ArcGIS software were used to change additional map elements legends etc. The final map was exported as a JPEG file.

Determining Occurrence and Density of Z. bicolarata

Quadrate sampling was performed randomly to determine the occurrence, intensity, and density of the *Z. bicolarata* in high-density Parthenium-reported DS divisions of the Jaffna as per the Parthenium distribution map reported by Kishojini *et al.* (2018), and opportunistic observations. The same methods were also used in other districts too (Shrestha *et al*., 2019). Readings were taken by throwing a 5 m^2 plastic quadrate. Each sampling point was replicated five times to minimize the error. For the calculation of the density of *Z. bicolarata* following formulas were used (Raunkaier, 1934; Stromberg, 1993).

Damage Incidence of Z. bicolarata on Parthenium

Damage incidence of *Z. bicolarata* on Parthenium was assessed by a scale of 0–5 using the standardized visual scorecard (Figure 01), and the degree of damage was calculated as per the level of damage inflicted by the *Z. bicolarata* as shown in Table 01 in the quadrate trapped sample.

$$\text{Absolute density(AD)(\%)} = \frac{\text{Total No of individuals of a species in all Quadrates}}{\text{Total no of Quadrate}} \times 100\ \%$$

$$\text{Relative density (AD)(\%)} = \frac{\text{Absolute density for a species}}{\text{Total Absolute density for all species}} \times 100\ \%$$

Measuring of Feeding Ability

Zygogramma bicolorata grub's and adult beetle's feeding ability was measured in a quarantine greenhouse at the Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, situated in the dry zone of Sri Lanka (Longitude: 80.4, Latitude: 9.32, Altitude: 46m) as per the procedure reported by Halangoda *et al.*, 2022. One-month-old Parthenium plant grown in pots as two plants per pot into the separate cages was inoculated with 0, 2, 4, 8, and 10 numbers of grabs (2nd & 3rd stage juveniles) and adults per cage, and replicated three times. Damage incidences were accessed every 24hrs until the score value was recorded as five (Table 01). The trial was conducted and maintained at room temperature of 29±2 °C at 70±5 % RH.

Experimental Design and Statistical Analysis

The greenhouse experiment was conducted using Complete Randomized Design (CRD). Analysis of variance (ANOVA) was performed in SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Tukey's HSD multiple comparison test was used to identify the best treatment combination at $P < 0.05$.

Table 01: Degree of damage of *Z. bicolorata* on *P. hysterophorus*

Levels of damage on *P. hysterophorus*	Score value
No defoliation	0
<10% of weed leaves defoliated	1
10 ->25% weed leaves defoliated	2
25->50% weed leaves defoliated	3
50->75% weed leaves defoliated	4
75-100% weed leaves defoliated	5

Figure 01: Degree of damage of *Z. bicolorata* on *P. hysterophorus* a) No defoliation; b) <10%; c) 10 ->25%; d) 25 - >50%; e) 50 - >75%; and f) 75 - 100% weed leaves defoliated

RESULTS AND DISCUSSION

Detection of Zygogramma bicolorata in Northern Sri Lanka

The mapping of the distribution of Parthenium in Northern Sri Lanka was started in 2011 and completely mapped in the year 2018 (Kishojini *et al.*, 2018), there were no single bio agents associated. In the year 2019 annual survey, unknown beetles were observed on almost all the Parthenium plants in Tellippalai (J/228) DS division, Valikamam north of Jaffna, Sri Lanka delimited by the North-South GPS range of 9.795139, 80.041215- 9.779417, 80.037729 and East-West GPS range of 9.795139, 80.041215- 9.783577, 80.024600 (Figure 02d and 03a). The beetles were collected and identified at the entomology laboratory of the Department of Agricultural Biology, Faculty of Agriculture, the University of Jaffna in Sri Lanka. The adult was 6.5 mm to 7.0mm in length, oval-shaped, convex dorsally, and flat ventrally (Figure 02d). The shiny light metallic brown elytra wings were marked with undulating black lines that ran longitudinally. The rest of the body and appendages were light to dark brown (Jayanth and Bali 1993; Javaid and Shabbir 2007). The above-mentioned morphological characters and feeding habits matched with previously reported morphometric characteristics of parthenium or Mexican beetle [*Z. bicolorata* Pallister (Coleoptera: Chrysomelidae)], so its identity was confirmed (Pakeerathan, 2019). In the 2020/2021 survey, all the life stages of the *Z. bicolorata* i.e. egg, grub, pupa and adult were observed (Figure 02a, b, c, and d). The morphological characteristics of the biological stages of *Z. bicolorata* exactly tally with the previous literature (Jayanth and Bali 1993; Siddhapara *et al.*, 2012; Parwinder and Maninder 2008).

Figure 02: Life stages of *Zyggramma bicolarata* a) Eggs; b) Grubs (Larva); c) Pupa and d) Adult beetles observed in Northern Sri Lanka (Photo: Pakeerathan, 2019)

Table 02: Distribution of *Zygogramma bicolorata* in Northern Sri Lanka

Districts (Northern Sri Lanka)	Number of DS divisions studied	Number of locations sampled		Opportunistic observations		Occurrences (%)	
		2019/2020	2020/2021	2019/2020	2020/2021	2019/2020	2020/2021
Jaffna	15	50	200	200	800	04	137
Kilinochchi	4	20	50	50	50	Nil	Nil
Mannar	3	20	50	50	50	Nil	Nil
Mullaitivu	5	20	50	50	50	Nil	Nil
Vavuniya	4	20	50	50	50	Nil	Nil
Total	30	130	400	400	1000	04 (0.75%)	137 (9.78%)

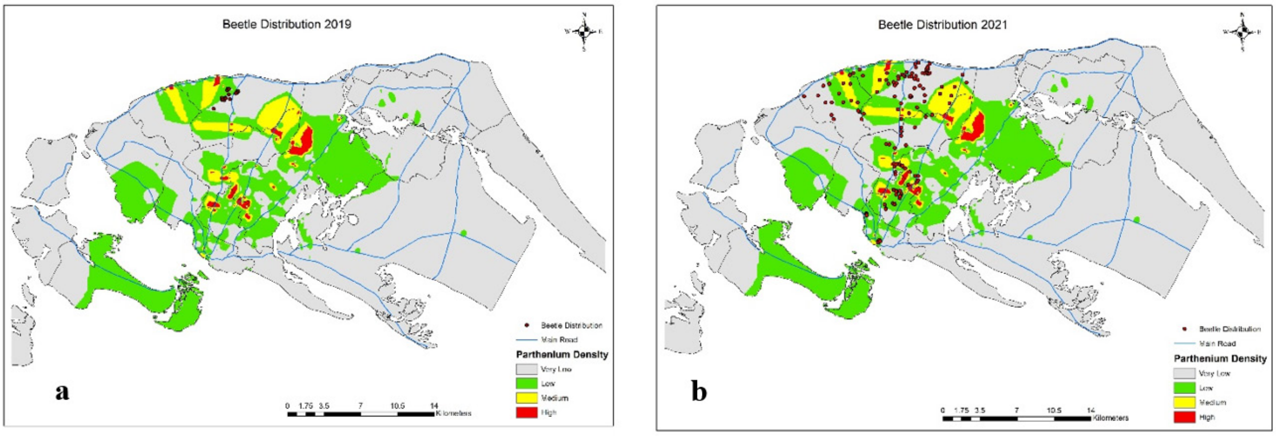

Figure 03: Distribution of *Zygogramma bicolorata* in Jaffna district a) in year 2019/2020; b) in year 2020/2021

Mapping of Distribution of Zygogramma bicolorata in Northern Sri Lanka

Identification and mapping of dispersal patterns of local biocontrol agents of any alien weeds is very important to manage or eradicate such invaders eco-friendly. Current survey findings exhibit that the potential Parthenium's biocontrol agent *Z. bicolorata* was reported only in the Jaffna district among all five districts surveyed in the years 2019/20 and 2020/21 (Table 02). In the years 2019/20, the distribution of the *Z. bicolorata* was mapped only in the Valikamam North DS division (Fig. 3a), and the density of the *Z. bicolorata* population was 0-12 per m^2 (Table 02). The number of occurrences of *Z. bicolorata* was four and 137 out of 530 and 1400 places sampled in the years 2019/20 and 2020/21, respectively. The locality percentage of *Z. bicolorata* distribution increased from 0.75% to 9.78% but was not represented evenly throughout Northern Sri Lanka.

In 2020/2021 exploration mapped the distribution of the *Z. bicolorata* population in Jaffna, Nallur, Valikamam East, Valikamam North, and Valikamam North-West DS divisions, out of 15 DS divisions surveyed (Figure 03b). The density of the *Z. bicolorata* population was 35-62 per m^2 in Valikamam North DS division, whereas 13-34 per m^2 in Valikamam East and Valikamam South, and 0-12 per m^2 in Jaffna and Nallur DS divisions, respectively (Table 03).

Table 03: Parthenium and its biocontrol agent's mean density and damage in Jaffna district

Number of DS divisions studied in Jaffna District	Parthenium density per m^2	*Z. bicolarata* density per m^2		Mean damage percentage
	2017/2018	2019/2020	2020/2021	2020/2021
Delft	0-12	0	0	No defoliation
Island North	0-12	0	0	No defoliation
Island South	0-12	0	0	No defoliation
Jaffna	13-34	0	0-12	<10%
Karainagar	0-12	0	0	No defoliation
Nallur	35-62	0	0-12	<10%
Thenmaradchi	13-34	0	0	No defoliation
Vadamaradchi East	13-34	0	0	No defoliation
Vadamaradchi North	13-34	0	0	No defoliation
Vadamaradchi South-West	13-34	0	0	No defoliation
Valikamam East	63-97	0	13-34	10 ->25%
Valikamam North	112-161	0-12	35-62	25->50%
Valikamam South	63-97	0	13-34	10 ->25%

Mapping of distribution and density of any living organism using GPS devices is a novel and emerging cost-effective technique. Current investigation mapped new Parthenium invaded areas in the Jaffna district of Sri Lanka apart from the earlier reports (Kavinthan, 2011; Kishojini *et al.*, 2018). The dispersal rate of Parthenium in the Valikamam north of Jaffna district is higher than the dispersal rate of *Z. bicolarata*, because of the public access of earlier declared high security no man zone for national security reasons, and now the unrestricted movement of vehicles with soil and other materials for construction of road and infrastructure. Moreover, soil and climatic requirement in Sri Lanka is highly optimal for the distribution and adaptation of Parthenium (Kriticos *et al.*, 2015). Shrestha *et al.* (2019) reported that road access appears to be the major pathway for the *Z. bicolorata* long-distance dispersal and it had spread 15.4% of the weed occurrence locations in Nepal and caused a low amount of damage.

The parthenium defoliator *Z. bicolorata* is a potential biocontrol agent and its presence was reported first time in the Jaffna district of Northern Sri Lanka in 2019 only in Valikamam North (Pakeerathan, 2019), but in the current survey, *Z. bicolorata*'s distribution has been mapped in many localities in the same district. This is a good indication that the biocontrol agent is adapted to the invaded new area, but dispersed slowly.

Dhileepan and Wilmot Senaratne (2009) reported that *Z. bicolorata* was brought from Mexico through a classical biocontrol program and introduced to South Asian countries like India, Sri Lanka, Pakistan, Nepal and Bhutan in 1984, namely. However the adaptation of *Z. bicolorata* was successful in India and widespread in southern India (Viraktamath *et al.*, 2004). This gives us evidence that the *Z. bicolorata* invaded by passing across the border from southern India to Sri Lanka and adapted to survive in the invaded environment and dispersing in its preferred way. From the Punjab province of India, *Z. bicolorata* invaded Pakistan in 2006 passing the border (Javaid and Shabbir, 2007). Jayasuriya (2005) reported that the Parthenium biocontrol agents such as the stem-galling moth *Epiblema strenuous* (Walker) and the summer rust *Puccinia* *xanthii* var. *parthenii-hysterophorae* (previously known as *P. melampodii*) were imported from Mexico to Sri Lanka in 2003, and after completion of quarantine procedure, *E. strenuous* was field released in 2004, but has not been observed so far in the dry-zone of Sri Lanka (Dhileepan *et al.*, 2009). Efforts were made in 2003 to introduce the leaf-feeding beetle *Z. bicolorata* into Sri Lanka, but, were not successful due to failure to rear a culture under quarantine conditions. The occurrence of heat waves may influence the performance and survival of *Z. bicolorata*, potentially impacting its field establishment and effectiveness as a biological control agent (Chidawanyika *et al.*, 2017). Several studies showed that mass multiplication of biocontrol agents was saucerful under laboratory optimal environments, failed to perform under real field thermal conditions due to the climatic variations (Afaq *et al.*, 2021). This is observed in the current study because the *Z. bicolorata* detected area is hot, therefore, its migration and reproduction were low in the hot and dry period of 6-7 months and started multiplication and dispersal in the *Maha* season (Chidawanyika *et al.*, 2017).

Field Observation of Zygogramma bicolorata Feeding Efficiency

It was observed that adults and grubs of *Z. bicolorata* consume Parthenium leaves at all growth stages and defoliates the plants in varying intensity. In 2019/2020, the adult stage of *Z. bicolorata* was the only damaging stage and was reported with few clusters of egg masses. The mean damage percentage was <10% in Valikamam North. In 2020/2021, it was recorded that the adult beetles, and 2nd and 3rd stage grubs were the voracious feeders as groups. The damage incidence varied from <10%-100% (Figure 04), but the maximum mean damage was 25->50% in Valikamam North and 10->25% in Valikamam East and Valikamam South (Figure 04d). Whereas in other all *Z. bicolorata* reported areas, the mean damage percentage was <10% (Figure 04b).

Beetle-damaged plants were mature and observed during the wet season in 2019, but in 2020/2021, all stages of Parthenium plants have been eaten away by the grubs and adults. The second and third prolific defoliators are capable of removing all parthenium leaf material from a stand. Complete defoliation and flower damage will lead to the wilting, dying, and suppression of the seed production Parthenium (Jayanth and Bali, 1993).

Greenhouse Feeding Efficiency of Zygogramma bicolorata

It was observed that the grubs were mainly fed the young leaves, and 8 grubs took 9±0.75 days to completely defoliate a Parthenium plant (Score-5) (Figure 05 and 06a). Whereas 8 adult beetles took an average of 11±0.62 days to make 75-100% damage incidence (Figure 06b). There was a significant difference between grub and adult damage or defoliation rate at $P < 0.05$. The feeding behavior of the adult beetles and the grubs were similar but score values were changed in every two days. In the adult beetle inoculated cages, the feeding rate was constant at 2nd-4th days and 7th-9th days in the adult inoculated cages. This may be the reason for the observation of frequent mating behavior. The copulation duration of *Z. bicolorata* is upto 60 minutes. A male riding on a female's back with his aedeagus inserted in the female genital tract is one of the most important factors influencing reproductive success (Bhaisare *et al.*, 2021). Further Kumar (2019) reported that food consumption and growth rates of unmated *Z. bicolorata* adults were higher than mated adults. This evidence supports why the feeding score was constant because during mating both male and female adults will not eat.

Figure 04: **Degree of damage of *Z. bicolorata* on *P. hysterophorus* a) No defoliation; b) <10%; c) 10 ->25%; d) 25->50%; e) 50->75%; and f) 75-100% weed leaves defoliated**

Siddhapara *et al*. (2012) reported that the 2nd and 3rd grub stage of the *Z. bicolorata* was a voracious feeder on young leaves and active during feeding and the older instars damaged all plant parts leaving behind midribs. Cowie *et al*. (2019) reported that *Z. bicolorata* larval feeding is significantly affected by the environmental conditions, and the quality of *P. hysterophorus*. The per day consumption of adult beetles was significantly less in comparison 2nd and 3rd instar grubs (Bhumannavar and Balasubramanian 1998). Feeding efficiency is an important factor in deciding which stage of the bioagents significantly contributes to the reduction of weed biomass.

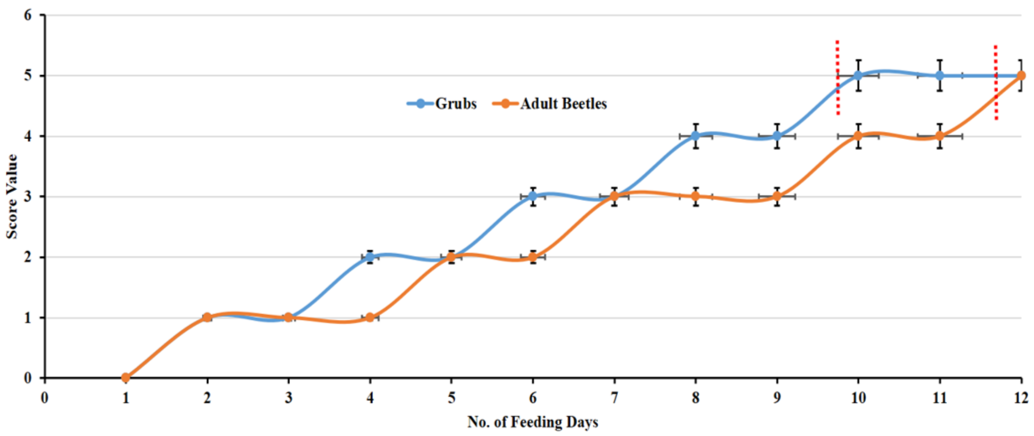

Figure 05: **Feeding rate of *Zycogramma bicolarata* grubs and adults on Parthenium plants**

Figure 06: Damage on *Parthenium Hysterophorus* By A) Grubs; B) Adult Beetles of *Zygogramma Bicolorata*

Parthenium is a noxious alien weed of national significance. Monitoring of dispersal of invasive weed into the new area, and biological agents associated with the invader are crucial to planning and implementing the best management strategies to manage the weed ecofriendly and to conserve the potential biocontrol agents, respectively.

In 2019, Jaffna International Airport in Sri Lanka was opened for international flight services from the closest airports in southern India such as Thiruchchi, Chennai, and Mathura. The site where *Zygogramma bicolorata* was first detected (Valikamam) is approximately 5 kilometers from the Jaffna International Airport. Therefore, it is suspected that *Zygogramma bicolorata* is a very recent introduction to Sri Lanka from southern India (Pakeerathan, 2019).

The annual mean temperature in Jaffna is approximately 29^{0}C. Such temperatures will further facilitate the population increase of this beetle. Dhileepan and Wilmot Senaratne (2009) reported that *Z. bicolorata* was not in Sri Lanka despite a climate suitable for its growth. Halangoda *et al*. (2022) reported that pesticides such as Carbosulfan 200 g/l SC and Abamectin 18g cause the highest mortality in grubs and adult beetles, even at the recommended dose. Therefore, selective use of chemicals would be ideal to minimize the impact on *Z. bicolorata*. The accidental introduction of *Z. bicolorata* into the country is a good indication that it can control the Parthenium as early as possible if the anthropogenic activities favor its survival.

The current research finding would significantly contribute to executing the bio-controlling of Parthenium in Sri Lanka.

CONCLUSION

This investigation reports the first finding of the invasion of *Z. bicolorata* into Northern Sri Lanka. GIS-based mapping exhibits and predicts that the *Z. bicolorata* has successfully colonized the Parthenium-invaded areas and inflicted the maximum damage incidence of 25->50% in Valikamam North of Northern Sri Lanka. The greenhouse studies conclude that 8 grubs needed (2nd-3rd juvenile stage) 9±0.75 days for the destruction of a Parthenium plant. The invasion rate of *Z. bicolorata* into the Parthenium existing area is very minimal. Augmentative release of *Z. bicolorata* is recommended to achieve successful parthenium management.

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Declaration

The authors declare that the research data described in this manuscript has not been published anywhere

Conflicts of Interest

The authors declare no conflict of interest

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