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# Biological control of Parthenium in India: status and prospects

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## ABSTRACT

***Parthenium hysterophorus* L., commonly known as carrot weed or congress grass in India has been considered as one of the worst weeds responsible for causing health problems in men and animals besides loss to crop productivity and plant biodiversity. The weed has infested about 35 million hectares of land in India since its first notice in 1955. Now it has become one of the main weeds in almost all types of agricultural lands besides infesting wasteland, community land, road and railway track sides and forests. In an attempt of biological control, search for suitable bioagent began in 1980s in India through systematic surveys. The work on biological control through competitive plants was started with the search of *Cassia sericea* from south India which lead to more attempts to manage Parthenium by competitive plant species. *Casia tora* and *C. sericea* have been recommended most suitable plant species for management of Parthenium in wasteland, on the raod side and community land. Likewise, in spite of hundreds of reports of pathogens causing disease on Parthenium, none of them has qualified as successful bioagent. So far, attempts of developing effective mycoherbicides have not yielded any success. Not even a single indigenous insect species has proved successful in spite of occurrence and infestation by many species. Under classical bilogical control of Parthenium in India, three insect species were imported in 1983 from Mexico, out of which only host-specific leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) was proved successful. *Z. bicolorata* has established in many parts of the country and has been found responsible to decrease the Parthenium densities in different parts of India. This paper gives details of current status of biological control of Parthenium and future strategies in India.**


**Key words:** Parthenium, Biological control, *Zygogramma bicolorata*, Compatetive plants, Pathogens on Parthenium.

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*Parthenium hysterophorus* L., (Asteraceae) is a weed of global significance occurring in Asia (Bangladesh, India, Israel, Pakistan, Nepal, southern China, Sri Lanka, Taiwan and Vietnam), Africa (Ethiopia, Kenya, Madagascar, Mozambique, South Africa, Somalia, Swaziland and Zimbabwe), Australia and the Pacific (New Caledonia, Papua New Guinea, Seychelles and Vanuatu (Dhileepan, 2009). It has been well established that Parthenium causes severe human and animal health issues, agricultural losses besides serious environmental problems like loss of biodiversity (Sushilkumar 2005). The weed was first reported in India in 1955 (Rao 1956) and now occurs throughout the country (Yaduraju *et al*. 2005) in about 35 million hectares of land Sushilkumar and Varshney 2007. *Parthenium hysterophorus* has also spread to neighboring countries like Pakistan (Javaid *et al.* 2005, Shabbir and Bajwa 2005), Sri Lanka (Jaisurya 2005), Bangladesh (Rahman *et al.* 2008, Karim 2009) and Nepal (Adhikari and Tiwari 2004).

Biological control of weed is the intentional manipulation of natural enemies by man for the purpose of controlling harmful weeds. Biological control does not advocate complete eradication of the unwanted organism, but rather mean to maintain its population at lower than average that would occur in the absence of the bio- control agent. Insects, fungi, nematodes, snails, slugs, competitive plants and microorganism may be bio-control agents for Parthenium. So far in the world, insects have received maximum attention in biological control of Parthenium followed by pathogens and competitive plants.

Singh (1997) considered use of biological control agents and exploitation of competitive plants, the most economic and practical way of managing Parthenium. During last few years much emphasis has been given to control Parthenium through various biological agents like insects, pathogens and competitive plants. In past, attempts were made to review work on biological control of Parthenium in context to India, Australia and global situations (Singh 1989, 1997, Sushilkumar 1993, Sushilkumar and Bhan 1995a, McFadyan 1992, Dhileepan and McFadyen 1997, Evans 1997, Dehileepan 2009).

Biological control of Parthenium through insects, pathogen and competitive plants gained momentum in India in 1980s with publication of more reports about the indigenous bioagents infesting Parthenium. The classical biological control was started with the introduction of a host-specific leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) from Mexico (Jayanth 1987). In spite of good information available on Parthenium about insects, fungi and plants infesting it, countable efforts were made in past to review all such information at one place. This paper gives the current scenario of different group of bioagents and their present status in controlling Parthenium in India along with the recent important work carried out on this aspect in the world.

**Biological control through competitive plants**

Biological control through competitive plants can be achieved either by conservation of naturally occurring plant species or through deliberate use of known competitive plant species to suppress the growth of the Parthenium. Most of the work on this approach has been carried out in India and now gaining momentum in other countries too.

***Biological control through competitive plants in India***

First time Maheswari (1966) reported a wasteland weed *Xanthium strumarium* to compete with Parthenium. Singh (1983) reported that *Cassia sericea* (= *C. uniflora*) compete and suppress Parthenium and may be used for its biological management. Mamtha and Mahadevappa (1988) found that Parthenium did not establish in the area where natural vegetation was not uprooted. It was reported that in Hoobli city of Karnataka state of India, the Parthenium remained a problem when other vegetations were removed in an effort to control the weed in the region but in Dharwad (Karnataka) where such vegetation removal was not done, Parthenium remained in low state of infestation (Mahadevappa 1999). In a survey, Mahadevappa and Ramaiah (1988) identified many plant species which competed with Parthenium. They found that Parthenium plants which grew along with *C. sericea* were weak and having low dry weight while Parthenium was having good height and high dry weight when it was found alone. Mahadevappa (1996, 1997, 1999) considered *Cassia sericea* as potential competitive plants to replace Parthenium. An another weed of this family, *Cassia tora* was found to replace Parthenium naturally in Madhya Pradesh (Sushilkumar and Bhan 1997a).

In Madhya Pradesh, the use of marigold was also advocated to suppress the growth of Parthenium in the protected areas (Kauraw *et al.* 1997). The broadcasting of seeds of *C. tora* before monsoon on the infested site of Parthenium reduced the Parthenium intensity by 95% (Tiwari *et al.* 1997). In Madhya Pradesh, heavily infested Parthenium sites were replaced at many places by deliberate broadcasting of seeds of *C. tora* during March-April Sushilkumar and Varshney 2007). In Chhatisgarh, Kolhe (2006) identified *Malva pluciea*, *C. tora* and *C. oxydentalis* to suppress Parthenium while in Kerala, Abraham and Girja (2005) reported *Chromolaena odorata, Cassia tora, C. oxydentalis* and *Sida aquata* suppressing Parthenium. In Delhi, Gautam *et al.,* (2005a) identified about 23 plant species suppressing Parthenium. They advocated deliberate use of *Kochea indica* for replacement of Parthenium in the area. In our view, the use of this species has yet to be evaluated as this plant species may soon enter into crop fields owing to its tiny seeds which are produced in thousands of number by a single plant.

In Maharashtra, *Cassia tora, Hyptis suavelones, Tephrosia purpurea, Xanthium strumarium etc.,* were found to compete with Parthenium ( Sarkate and Pawar 2005, Gaikwad 2006). In Jammu &Kashmir, *Cenchrus* hybrid, *Vetiveria zijanioichrus* and *Symbopogon* were found compatetive species to overcome the growth of Parthenium besides *Leucenia leucocephala* and Pinus species (Singh and Kaur 1997). Under a co-ordinated project sponsored by Department of Biotechnology (DBT), Yaduraju *et al*, (2005) found many competitive plant species from different climatic zones of India like *Cassia tora*, *Croton bonapladanium, Croton sparsiflorus, Cannavis sativa, Hyptis suaveolens, Amaranthus spinosus, Sida acuta, Tephrosia purpurea, Stylosanthes scabra, Cassia auriculata, C. obtusifolia etc.*

***Cassia tora use in competitive biological control:*** On the basis of available information about competitive plants spices, it can be concluded that among all the plant species, *Cassia tora* can be recommended for deliberate replacement of Parthenium on the road side, community and waste lands in most of the states of India on the basis of its low seed productivity and spread ability and of natural occurrence in wide climatic range of India. The seeds of *Cassia tora* are heavy in weight so there are least chances of its spread from the infested sites to crop fields. The Parthenium infested area may easily be replaced with *C. tora* by broadcasting of its seeds during March- April at the rate of 40-60 kg/ha. The broadcasted seeds germinate on the onset of monsoon and overcome Parthenium growth which also germinates along with *C. tora*.

***Biological control through competitive plants in some other countries***

In other countries little work has been done on biological control by competitive plants however, in Australia, some plant species namely *Bothriochloa inschupta* (blue grass), *Decanthim aristatum* (floren blue grass), *Cenchrus ciliaris* (bafel grass) and *Clitorea ternatea* (butterfly pea grass) were found to suppress the growth of Parthenium O'Donnell and Adkins (2005). In USA, *Sorghum halepanse* (Johnson grass) *Impereta cylindrica* (cogan grass) *Echinocloa crusgali* (barniyard grass), *Ipomoea* species, *Senna obtusifolia* and *Ambrosia trifid etc*, were found dominating on wasteland, community and agriculture land instead of Parthenium (Reddy and Bryson 2005). Plant species like *Eragrostis curvula, Panicum maximum and Digitaria eriantha* from South Africa (Van der Laan *et al.*, 2008) and *Imperata cylindrica, Cenchrus pennisetiformis* and *Sorghum halepense* (Shabbir and Bajwa 2005, Javaid *et al*. 2005) from Pakistan have been reported to compete with the Parthenium.

**Biological control through microorganism**

The method by which the weeds can be managed through the use of pathogens is called biological control through pathogens. In this approach, fungi, bacteria and viruses may be used. The toxins produced by these organisms which may kill the weeds are known as bioherbicides and toxin produced by the group of fungi are called mycoherbicides. Among different microorganisms, maximum work has been done on fungi. Use of pathogens and mycoherbicides against Parthenium has been reviewed by Evans, (1997), Sreeramkumar (1998), Sreeramkumar and Evans (2005) and Sushilkumar and Varshney (2007). In a survey done in Mexico, Argentina, Trinidad and Cuba during 1983-84 and 1995-97 by the scientists of International Institute of Biological control (IIBC), about 26 species of fungi were recorded on Parthenium out of which rust *Puccinia abrupta* variety *parthenicola, P. melampodi* and smut *Entyloma compositarum* were thought to be suitable for biological control purpose. There are many hosts of *P. melampodi* but Seier *et al*., (1997) searched host specific strains of this fungus on Parthenium. *Parthenium abrupta* and *P. melampodi* have been released in Australia but were not found to cause appreciable damage to Parthenium (Evans 2001). Jaisurya (2005) found *P. melampodi* to infest Parthenium in Sri Lanka. The work carried out by different workers in different parts of world on host specific Pathogen on Parthenium are given (Table 1).

**Table 1. Host specific fungi reported on Parthenium**

| Pathogenic fungi | References |
|---|---|
| *Puccinia abrupta* variety *parthenicola* | Parmeli 1997, Parker *et al.* 1994, Evans 1997, Fuzi *et al.* 1999. |
| *Puccinia. melampodi* | Parmeli 1997, Parker *et al.* 1994, Evans 1997, Fuzi *et al*. 1999, Jaisurya (2005) |
| *Entyloma compositarum* | Ciferri 1963, Evans 1997, Seier *et al.*, (1997) |
| *Cercospora parthenii* | Chupp 1956 |
| *Odium parthenii* sp. Nov. | Satyaprasad and Usharani 1981 |
| *Fusarium pallidoroseum* | Kauraw *et al.* 1997 |

***Biological control of Parthenium through fungi***

***From Andhra Pradesh:*** Some pathogens have been reported to attack Parthenium (Luke 1976, Kalidas 1981). Kumar and Rao (1977) observed a leaf spot disease caused by *Colletotrichum gleosporioides* (*=Clomerella cingulata*) and Rao and Rao (1979) reported *C. capsici* in Andhra Pradesh. Another fungal disease *Exserohilum rostratum (=Setosphaeria rostrata*) was reported from the same area (Rao and Rao 1987). Satyaprasad and Usharani (1981) reported powdery mildew causing *Oidium parthenii* on parthenium in Osmania University campus, Hyderabad and suggested that pathogen can be exploited against Parthenium under the biological control programme. Bioherbicide prepared from *Alternaria alternata* was found effective against seedlings of Parthenium by Despandey *et al*. (1982).

***From Tamil Nadu :*** Kumar *et al.* (1979) reported a few plants of Parthenium in advanced stages of wilting in North Arcot district and found to be infected with the root rot fungus, *Rhizoctonia solani.* The epidemiology and host-range of *Odium parthenium* were investigated by Manickam *et al.* (1997) at Coimbatore. They inoculated *O. parthenii* artificially on *Helianthus annuus* (sunflower), *Cucumis sativus* (cucumber), *Cucurbita moschata* (pumpkin), *Vigna mungo* (blackgram), greengram and *Sesamum indicum* (sesame) and found to be non-infective to the plants. Jeyalakshmi *et al.* (2005) during survey at Coimbatore recorded 21 pathogenic species on Parthenium. They found *Lasidiplodia theobromee,* a host specific blight causing fungus which causes severe damage to Parthenium at 15-30 days stage. They also reported *Oidium partheni* to cause severe damage on Parthenium at flowering stage.

***From Madhya Pradesh:*** Rajak *et al.* (1990) listed 25 species of fungi isolated from infected/infested parts of Parthenium plants from different places in and around Jabalpur (Madhya Pradesh). The species of *Alternaria, Colletotrichum, Drechslera, Curvularia, Phoma, Acremonium, Myrothecium, Cladosporium,* associated with leaf spots and species of *Chaetomium, Aspergillus, Rhizopus*, *etc* were found associated with seeds or floral parts of the weed. However, the researchers did not ascertain the pathogenicity of the fungi. Pandey *et al.* (1990) came across a blight disease of Parthenium and the casual organism was isolated and identified as *Myrothecium roridum*. Later, the same group evaluated this pathogen for its efficacy. Pandey *et al.* (1991) assessed the pathogenicity of 19 fungi and concluded that *C. gleosporioides, Alternaria alternata. A. dianthi, A. macrosporus, Myrothecium roridum, Fusarium oxysporum, F. nioniliforme, Phoma herbamm and Bipolaris* sp. were able to cause considerable damage to the weed under laboratory conditions. *Fusarium oxysporum* and *R. solani* were evaluated by Pandey *et al.* (1992b). Both the pathogens were found to be highly effective, causing severe infection and significant damage to the weed in laboratory conditions. The possibility of controlling the weed with *Sclerotium rolfsii* was studied by Pandey *et al.* (1996, 1998). They could obtain 90-95% and 35-40% mortality of Parthenium seedlings in the greenhouse and field trials, respectively. Subsequent tests for host-specificity, two isolates of the pathogens in pot and field experiments showed only a limited number of cultivated species to be susceptible. They suggested to use isolates as potential mycoherbicides. The factors affecting the viability and virulence of *S. rolfsii* were also investigated. Histopathological investigations revealed that the pathogen penetrated the host through mass action and production of oxalic acid (Mishra *et al.* 1996). Chand *et al.* (1995) recorded an new species *Xanthomonas campestris* pv. *partthenii* pathvar nov. on Parthenium.

In efforts of biological control of Parthenium through microorganism, Kauraw *et al.* 1997, Bhan *et al.*1998) reported occurrence of fungi *Fusarium pallidoroseum, Colletotrichum gleosporiodes, Alternaria alternata, Sclerotium rolfsii* and *Sclerotinia sclerotiorum* on Parthenium from Jabalpur (Madhya Pradesh) among which strain of *F. pallidoroseum* was reported to be host specific. In field evaluation trial, *F. pallidoroseum* was found to reduce seed germination, seedling vigour, height of plant, number of branches and number of flowers. *Alternaria alternata* was found to infect leaves, branches and flowers of *P. hysterophorus*. This pathogen was considered to be a potential biocontrol agent for Parthenium management. Spray of fungal suspension of another fungus *Sclerotinia sclerotiorum* (200g mycelium/ litre water) could also reduce the vigour of Parthenium. Spray of spore suspension $10^7$ spores/ml water of *Trichoderma viride* could reduce height, no. of branches/ plant and no. of flowers/plant in plots sprayed at 30 DAS. Gyatri and Pandey (1997) reported leaf spot causing fungus *Colletotrichum dematium.*

***From Karnataka:*** Another powdery mildew fungus, *Oidium parthenii* and a rust fungus *Puccinia abrupta* var. *parthenicola* were also proved to be highly pathogenic (Sreeramkumar 1998). Siddaramaiah *et al.* (1984) observed *S. rolfsii* causing wilting and death of Parthenium plants in and around groundnut plots at Dharwad. Work at Project Directorate on Biological Control (PDBC), Bangalore, India with locally isolated *S. rolfsii* showed the potentiality of the pathogen to bring about total kill of young Parthenium plants within six days of application with 4-5 sclerotta per plant (Sreeramkumar 1998). The efforts were made to develop bioherbicde formulations to control Parthenium from the host specific strain WF (Ph)30) of *Fusarium pallidoroseum*. Parthenium was found very susceptible against this strain in laboratory but not in the field conditions. (Sreeramkumar and Evans 2005).

***From Delhi and adjoining states:*** Work with *Alternaria alternata* suggested the incapability of the pathogen to cause substantial damage to host tissues and failed to qualify as a potential bioagent for suppression of the weed (Dhawan and Dhawan 1995). *Curvularia lunata* (=*Cochliobolus lunatus*) causing a leaf spot disease on Parthenium was described as a new host record by Aneja *et al.* (1994). Dhawan and Gupta (1997) isolated a total of 16 fungal species belonging to 10 genera from the spermoplane of Parthenium. *Alternaria alternata* occurred most frequently (30.33%) and *Epicoccum* sp. the least (00.16%). All the isolated fungi were considered weak pathogens except *A. alternata* which caused a minor leaf spot disease. During a survey at Udaipur (Rajasthan), Sharma and Gupta (1998) reported first time a fungus *Alternaria zinniae* as a leaf spots on Parthenium.

***Pathogen attacking both Parthenium and crops***

There are many reports from different workers about the pathogen attacking on Parthenium but the same pathogen were also reported to cause damage to crops. (Sushilkumar and Varshney 2007) (Table 2).

**Table 2. Pathogens which attacks Parthenium and crop both**

| Pathogen | Reference |
|---|---|
| *Sclerotium rolfsii* | Pandey *et al.*1996, Awadhya and Sharma 1997, Siddaramaiah *et al.* 1984 |
| *Myrothecium roridum* | Pandy *et al.* 1990 |
| *Colletotrichum dematium* | Gyathri and Pandy 1997 |
| *C. gloeosporioide* | Kumar and Rao 1977 |
| *C. capsici* | Rao and Rao 1979 |
| *Dreshlera australiensis* | Jayalakshmi *et al.* 2004 |
| *D. hawaliiersis* | Jayalakshmi *et al.* 2004 |
| *Alternaria alternata* | Dhawan and Dhawan 1995 |
| *A. zinnie* | Sharma and Gupta 1998 |
| *Fusarium oxysporum* | Pandy *et al.* 1992 |
| *F. solani* | Pandy *et al.* 1992 |
| *F. semitectum* | Rao and Rao 1987 |
| *Lasiodiplodia thiobromee* | Kumar and Singh 2000 |
| *Phoma sorgine* | Kumar and Kumar 2000 |
| *Curvularia lunata* | Aneja *et al* 1994 |
| *C. palesens* | Jayalakshmi *et al.* 2004 |
| *C. verruculsa* | Jayalakshmi *et al.* 2004 |
| *Eriysiphe cichoracearum* | Aneja and Khan 2000 |
| *Rhizoctonia solani* | Kumar *et al* 1979 |
| *Sclerotinia sclerotiorum* | Ghasolia and Shivpuri 2004 |
| *Syncephalastrum raceinosum* | Jayalakshmi *et al.* 2004 |
| *Exerohilum rostratum* | Rao and Rao 1987 |
| **Bacteria** | |
| *Ralstonia solanacearum* res-1 | Kishun and Chand 1988 |
| *Xanthomanas campestris* pv. *phaseole* | Ovies and Laranga 1988 |
| **Viruses** | |
| Tomato leaf curl virus | Rammappa *et al*, 1998 |
| Tobacco leaf curl virus | Valand and Muniyppan 1992 Swansan *et al.* 1998 |
| Tobacco streak virus | Rao *et al* 2003 |
| **Phytoplasma** | Mathur and Muniyappa 1992 Verma *et al.* 1974 |

On the basis of cited literature, it can be concluded that in spite of occurrence of several potential fungi on Parthenium and showing damage potential in laboratory conditions, so far non of the pathogens has shown promising results to suppress Parthenium in the natural field conditions in India.

### *Pathogenic bacteria on Parthenium*

Two types of bacteria namely *Ralstonia solanacearum* and *Xanthomanas campestris* were found attacking Parthenium. These type of bacteria generally infest seeds through roots. *Ralstonia solanacearum* was found to infest tomato, brinzal and potato crops besides Parthenium and may remain alive in the soil up to six months (Kishun and Chand 1988). *Xanthomanas campestris* was found to infest pulses like mung or green bean (*Vigna radiata* ) and urad (*Vigna mungo)* along with Parthenium.

### *Pathogenic virus on Parthenium*

Tomatoo lef curl virus have been found to infest Parthenium naturally due to which infected plant become stunted and leaf margin rolls either inward or upward and become stiff with yellowish margin. Its leaves are thicker than normal, with leathery texture. The young leaves have yellowish color, cupped, thick, and rubbery. It's transmitted by sucking insects of tobacco whitefly, *Bemisia tabaci* (Devraja *et al*. 2005). Tobacco streak virus has been found to attack Parthenium beside many crops like sunflower, soybean, tobacco, groundnut, urad, cotton etc. The thrip species of insects have been found to transmit the disease from Parthenium to crops.

### *Pathogenic Phytoplasma on Parthenium*

These are obligatory pathogen which need host for their survival. Phytoplasma type organism cause disease like pear decline, phyllody, aster yellow, potato witch broom, brinjal little leaf and Parthenium phyllody (Singh and Singh 1996). In India Pathak *et al,* (1975) reported first time phyllody disease on Parthenium. Aster yellow disease caused by Phytoplasma in Parthenium was demosntated by Raj *et al* (2002) by direct and nested polymerase chain reaction using universal primers specific to 16SrRNA gene of Phytoplasma. In Banguluru in 1988, Parthenium phyllody was observed in epidemic form varied from 20 to 75% (Mathur and Muniyyappa 1993) which declined seed production drastically in Parthenium. In Jabalpur and some other parts of Madhya Pradesh, large numbers of Parthenium patches were found infested with phyllody. Taye *et al* (2002) observed phyllody disease in low to mid altitude areas (900-2350 m.s.l.) of Ethiopia with incidence up to 75%. The phyllody infected plants were characterized by excessive branching, reduced plant height, leaf size, and transformation of floral structures in to leaf-like structures that lead to sterility. Janke *et al.* (2007) detected DNA fragments specific Phytoplasmas in *P. hysterophorus* as well as in important crops in Ethiopia, e.g. groundnut, sesame and grass pea. The Phytoplasmas belong to the Peanut witches´ broom (16 SrII) group transmitted by the leafhoppers *Orosius cellulosus* native to Ethiopia. Moreover, it could be shown that nymphs as well as adult plant hoppers of the genus *Hilda* (family Tettigometridae) collected from phyllody diseased *Parthenium,* can acquire these Phytoplasmas. This suggested that Parthenium represents a pathogen reservoir for the Phytoplasmas affecting agricultural crops in the country. Since Phytoplasma infections can lead to sterility of the inflorescence, severe losses in yield of agricultural crops could be expected. Thus, control of Parthenium and putative vectors transmitting phyllody disease is important.

## Biological control through insects

### *Biotic pressure on Parthenium by indigenous insects*

In India, many insects have been reported on Parthenium but none of the indigenous insect has been found host-specific yet. Among the insects, stem boring scolytid beetle, *Hypothenamus erudistus* caused wide spread damage to Parthenium. A cerembycid borer *Oberea* sp. was also found to kill this weed significantly (Kumar *et al.* 1979). *Earias* sp. used Parthenium as an alternate host (Thontadarya and Hiremath 1978). Common tailed mealy bug *Ferrisia virgata* (Char *et al.* 1975), mites *Tetranychus cucurbitae* and *Tetranychus* sp. (Puttaswamy *et al.* 1976*,* Rajulu *et al.* 1976) and insects *Aphis fabae* and *Pseudococcus* sp. were recorded on Parthenium ( Rajulu *et al.* 1976). Kumar *et al.* (1979) in a survey in Tamil Nadu at 104 places during October 1975 to October 1979, collected many insects, a mite and a few diseases on Parthenium. Severe attack of a bug *Leptocentrus taurus* and a scale insect *Orthezia insignis* was reported in Mysore and Bangalore, respectively (Thangavelu 1980*,* Srikant *et al.* 1988). Many other insects like mealy bugs, aphids, *Heliothis helicoverpa, Clania cramari, Dicrasia obliqua* and grass-hoppers have been reported feeding on Parthenium (Sushilkumar, personal observatiosn). Parthenium has been reported a alternate host for mealy bug *Ferrisia virgata* in Punjab and Haryana has became a nuisance for the cotton crop in the states. Some times, indigenous insects may also play important role in exotic weed suppression. A cerembycid borer *Nupserha* sp. has been found infesting Parthenium at Jabalpur and Vindhyanagar (M.P.). Survey revealed that the insects were capable of doing widespread damage (5-95%) to Parthenium but infestation varied place to place. (Sushilkumar, 1998b).

### *Introduction of* Zygogramma bicolorata *from Mexico in India and subsequent work on its different aspects*

Studies carried out by the Department of Land, Queensland, Australia in collaboration with the Commonwealth Institute of Biological control (CIBC) in Mexico showed that a large number of insects feed on Parthenium. Further survey work for three year in Monterrey (Mexico) resulted a total 159 species of phytophagous insects on Parthenium beisdes many unidentified species (McClay, 1980). Among these, many species were found host specific. Based on this survey, about nine insect species were introduced in Australia for biological control of Parthenium. Out of these, many species have been well established in Australia. Based on the success in Australia, three insects namely defoliating beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), the flower feeding weevil *Smicronyx lutulentus* Dietz (Coleoptera: Curculionidiae) and the stem boring moth *Epiblema strenuana* (Walker) (Lepidoptera: Tortricidae) were imported in India in 1983 to 1985 (Singh 1997). *S. lutulentus* could not be multiplied in the laboratory while *E. strenuana* was found to complete its life cycle on a oilseed crop niger (*Guizotia abyssinica* L. (Asteraceae), hence, its culture was destroyed (Jayanth 1987) in spite of the fact that this insect was considered to be a potential biocontrol agent in Australia (McFadyen 1985). Detailed host-specificity test of *Z. bicolorata* in Mexico (McClay 1980) and Austalia (McFadyen 1980, 1985) revealed it a safe bioagent.

***Host specificity test in India :*** On 21st August, 1983, a shipment of only 66 live beetles out of 307 sent by Dr. A.S. McClay of CIBC of Mexican sub-station was received at Bangalore. The beetles laid large number of eggs and good culture was built up for host specificity test. Detailed host-specificity tests under quarantine conditions confirmed the safety of *Z. bicolorata* to cultivated crops in the country. On 37 of 40 plant species tested, no adult feeding, oviposition or larval feeding were observed. Slight adult feeding was observed on jasmine (*Jasminum grandiflorum*) and niger (*Guizotia abyssinica*) but in multiple choice tests, oviposition and larval feeding occurred only on *P. hysterophorus*. *Z. bicolorata* reproduced under field conditions and caused considerable damage to *P. hysterophorus*. Based on these results permission of the Plant Protection Advisor to Government of India was obtained and field release was initiated in Bangalore in 1984 (Jayant 1987, Jayant and Nagarkatti 1987, Singh, 1997).

### *Controversy and its end over feeding of beetle,* Zygogramma bicolorata *on sunflower*

Seven years after first release of this beetle, it was found feeding on an important oilseed crop, sunflower (*Helianthus annuus*) (Sridhar,1991) followed by other reports (Kumar 1992). This started a tug of war and resulted the formation of a Fact Finding Committees (FFC) by Indian Council of Agricultural Research (ICAR) in November 1992 which suspended further intentional releases of the beetle till controversy is resolved. On FFC recommendations, ICAR funded a co-ordinated research project at 5 places in different parts of country Sushilkumar and Bhan (1995b). Research unequivocally proved that *Z. bicolorata* is a safe bioagent against Parthenium . Little feeding by the 'O' day beetles was attributed due to falling of Parthenium pollen on sunflower which attracted beetles to feed. Continuous feeding on sunflower caused degeneration of the ovary (Jayanth *et al*. 1997). Biochemical analyses conducted on grubs of *Z. bicolorata* reared on Parthenium and *Xanthium strumarium* indicated that body weight, total protein, free amino acid, DNA and RNA contents were all higher in the grubs reared on Parthenium , while moisture contents were similar. It was argued from these data that Parthenium was the most preferred host for *Z. bicolorata* (Sushilkumar and Bhan 1998, Sushilkumar *et al*., 1995, 1997a). Studies revealed that in spite of development of *Z. bicolorata* on sunflower for several generations in the laboratories, chances of beetle to become a potential pest was remote as the survival rate of larvae and beetles developed on sunflower was very low. The weight of male and female beetles was highest in Parthenium fed beetles followed by Xanthium and sunflower. Preference tests of grubs and adults also proved Parthenium as most suitable host. Maximum newly hatched larvae preferred Parthenium followed by Xanthium and sunflower (Sushilkumar 1998a). Further, it was established that *Xanthium strumarium*, acts as alternate host for *Z. bicolorata* (Sushilkumar and Bhan 1996) which confirmed the earlier report of Mexican beetle feeding on *X. strumarium* (Kumar 1992). On the FFC recommendations, Government of India lifted the ban imposed on the Mexican beetle in 1999. Now Mexican beetle can be multiplied and release anywhere in India for Parthenium suppression.

***Life cycle of* Z. bicolorata *in different parts of India***

Manyworkers have conducted detailed studies on life cycle of this bioagent at different parts of India (Table-3). The life cycle varied place to place and in different climatic conditions of India. Eggs are generally laid on the ventral surface of both young and old leaves. Hatching % has been reported differently by different workers ranging from 30 to 93% but Jayanth and Bali (1992) reported only 30-53% hatching of the eggs under laboratory conditions. Sushilkumar *et al.* (1997a) found more polyphenol content in mature leaves and presence of more mature grubs on old leaves which indicated more polyphenols requirement in old grubs. Bhumannavar and Balasubramanian (1998) found that third instar grubs and egg-laying females ingested maximum food.

The male and female may have a mean body length and weight of 5.6 and 6.5 mm and 30 and 40 mg, respectively. But they are easily differentiated by the shape of the last abdominal sternite as described by McClay (1980). The sex ratio is found to be in favour of females. Samples collected from the field at monthly intervals showed that the mean sex ration was 1 female: 0.40 males (Jayanth and Bali 1992). Other studies also found sex ratio in favour of female (Table 3). The males of *Z. bicolorata* may remain alive for longer period (122-271 days) while the females up to 109-198 (mean 129.30) days. Omkar *et al*. (2009) studied the effect of different constant temperatures on reproductive attributes of *Z. bicolorata*. They found that pre-oviposition and post-oviposition period declined with increasing temperature. Oviposition period decreased from 92.9 to 27.5 days with temperature increment after 27 °C.

It was reported earlier that *Z. bicolorata* produced an average of 836 eggs per female, with a maximum of 1786 (McFadyen and McClay 1981). Many workers in India representing different climatological conditions have studied the biology of the beetle and reported variation in the various biological parameters (Table 3).

**Table-3. Biological parameters of *Z. bicolorata* reported by different workers from India representing different climatological conditions of India**

| Biological attributes | Jayanth and Bali (1992) (Bangaluru) | Kulkarni *et al.* (1997) (Dharwad) | Sushilkumar (1998a) (Jabalpur) | Aherkar *et al.* (1992) (Prabhani) | Gautam (2002) (New Delhi) | Pandey *et al.*, 2001 (Haridwar) |
|---|---|---|---|---|---|---|
| Egg period (days) | 5 | 4-5 | 3-5 | 2-6 | 4-6 | 3-4 |
| Grub period (days) | 11-13 | 19-23 | 12-21 | 15-20 | 13-16 | 10-12 |
| Pupal period (days) | 10-12 | 7-10 | 5-11 | 10-12 | 5-6 | 8-10 |
| Egg to adult emergence period (days) | 27-29 | 30-38 | 20-37 | 27-38 | 23-27 | 20-28 |
| Egg group size ( nos. of eggs group) | 6 | NM | 3-5 | 4-5 | 1-4 | 4-6 |
| Egg laying/female | 1695-3360 | 684 | 550-960 | 551 | NM | 2500 |
| Hatching (%) | 30-53 | 85-91 | 45-93 | NM | 51-100 | NM |
| Pre-oviposition period (days) | 10-70 | NM | 6-14 | NM | 3-8 | 7-10 |
| Sex ratio (Female %) | 1:0.40 | 1: 0.70 | 1.0.60 | NM | 50-60 | NM |
| Male longevity | 122-271 | 31.97 | 62-110 | 30 | 35-90 | 120-240 |
| Female Longevity | 109-198 | 35.05 | 60-115 | 38 | 49-105 | 90-180 |
| Oviposition period | 89-138 | NM | 50-120 | NM | NM | 75-120 |
| Post oviposition period | 1-21 | NM | 1-8 | NM | NM | NM |

NM= Not mentioned

***Diapause behaviour in Z. bicolorata*:** Diapause is a unique feature in insects to avoid unfavourable conditions but adults of *Z. bicolorata* have been seen to undergo diapause in the soil under field conditions even under favourable conditions during August. The diapause behaviour of Mexican beetle is very interesting. As not all adults of *Z. bicolorata* entered diapause, it is debatable whether diapause in this insect can be categorized as 'obligatory'. However, since the surviving, adults perished in the field by February, without reproducing, they may not qualify for inclusion under 'facultative' category either. It was not clear what triggers diapause behaviour in *Z. bicolorata*, especially since abundant food is available and weather conditions remained favourable during July to September. Laboratory studies had indicated that diapausing adults of the beetle couldn't tolerate prolonged exposure to temperatures above $40^0$C (Jayanth and Bali 1993a, Virakatamath *et al.* 2004). Therefore, they were of the opinion that role of Mexican beetle in suppressing Parthenium may be limited in parts of northern and central India that experience high summer temperatures.

Sushilkumar (2005 and Sushilkumar 2008) has studied the diapause behaviour in detail. They found that adults of *Z. bicolorata* may enter over an extended period between July to December. In the climatic conditions of Jabalpur representing central India, it was observed that about 60% adult population of Mexican beetle developed during August died naturally by the end of November. Out of 40%, 30% population enter into soil below 2-6 cm, remaining 10% population remained without burying themselves into soil but their activity was very low. Generally, these beetles do not lay eggs and their movement also remains limited. In majority of the cases, such low activity-showing beetles became active with the increasing temperature in February March when they again start egg laying. A fraction of population of beetle may emerge from diapause at any time of the year depending on the micro-climate of the area but maximum diapaused population of adults emerged in May-June and June-July in south and north and central climatic conditions, respectively with the commencement of monsoon rains in the area. Soil moisture plays an important role in providing the conditions for diapausing or emerging from the diapause. Adults having inherent cues may enter in to diapause only once during its life time. The field collected population during June-July at Jabalpur consisted mainly of adults that had emerged after diapause, although the F1 generation was also represented during this period. Jayanth and Bali (1993b) in laboratory studies showed that adults younger than 15 days of age did not enter diapause. It was also observed that within a given generation, the percentage of adults entering diapause increased over time form 20% on the thirtieth day after emergence to 72% on the seventy-fifth day. They observed that percentage diapause increased over time, peaking at 72% during November. The onset of diapause would be strongly dependent on the age structure of the population at a given time. A detailed study conducted by Sushilkumar (2008) on diapause behaviour revealed that exposure of newly emerged adults to heat treatment of $35^0$C and to low temperature of $10^0$C could reduce diapause in *Z. bicolorata*. The low temperature can also be used as a medium for the storage of the mass reared beetles for a long time without having negative effect on their longevity and fecundity. They found that at $26^0$C, 63.8% of the beetles entered diapause, 13.3% did not diapause and 23% died by 75 days while due to initial heat treatment ($35^0$C), only 1.8% of the beetles entered diapause but mortality was high (57.0%) in 60 days. About 9.8% of the adults entered diapause when kept constantly at low temperature ($10^0$C) and there was lower mortality (3.5%) among the beetles compared to the beetles kept at higher temperature.

It was found that a delay of rainfall for more than 45 days could reduce the emergence of adults from diapause significantly. In such areas, new releases may have to be carried out for effective control of the weed (Visalakshy *et al*. 1998). Soil moisture played an important role in providing the conditions for burrowing or emerging from the diapause chambers. The studies also showed that diapausing adults had to be exposed to the high summer temperatures for diapause termination. It was possible to break diapause by continuous exposure to 30, 35 and $40^0$C for 22, 9 days and 10 hours, respectively, during February-March at Bangaluru. It was concluded that this method can be used to initiate mass multiplication for carrying out releases early in the season (Jayanth and Bali 1993b).

Jayanth and Bali (1992) reported that maximum percentage of diapause (40%) in laboratory reared insects in the F1 generation during November whereas 72% of the adults collected from the field during the same period entered diapause. Adults of *Z. bicolorata* were observed to mate before and after diapause. Gautam *et al*. (2005b) have studied and discussed the changing status of dispause in *Zygogramma bicolorata* in north India. Tewari and Kaur (1999) viewed that diapause in *Z. bicolorata* enables conservation of the beetles in a particular area during their migration, *i.e.*, diapause enhances the capability of *Z. bicolorata* as a biocontrol agent while the beetles shift from one area of attack to a new area and during this process of migration, the adults that have already undergone diapause are left behind while the rest migrate. The diapausing beetles on their emergence during the next monsoon, check the growth of the reoccurring weed of the area. Contrary to this, Sushilkumar *et al.* (2005c) argued diapause as an disadvantage to the bioagent.

***Population dynamics of*** *Z. bicolorata :* In general *Z. bicolorata* remained active in field between May and October every year in Bangalore conditions representing very congenial climatic conditions for the beetle. But in central and north climatic conditions, the beetle remain most active during July to September with some activity during February-March. At Jabalpur, all the stages of beetles were also recorded from the field in extreme winter and summer season near the high moisture regime. (Sushilkumar 2005, Dhiman and Bhargawa 2005).

In south, central and north India, population density of beetle was recorded highest in the month of August and September and lowest in the month of December and January. In central and north India, by the end of October, temperature of the region began to decline, which is correlated with the decline of Mexican beetle population drastically. However, a fraction of population, although in very low frequency, always occurred in the area, particularly near the places of water sources where Parthenium plants got opportunity to grow. During March-April, all the stages of *Z. bicolorata* were recorded indicating that *Z. bicolorata* began to build up its population from the March with the increase in temperature, which give opportunity to diapausing adult to emerge and start development. After March, again temperature increase resulting decline in humidity and correspondingly, decline of the beetle population that was built during the mild temperature of March. Population was recorded even in the hottest months of May and June when temperature touched about $45^{0}$C during day time when it was very torturous to stand in the direct sunlight in the open field. It was seen that adult beetles also hide themselves amidst the curly bunch of the old leaves of the plants during day time in May and June. During lean season that is November to April, beetles were found only on succulent small plants having rich green foliage and were devoid of inflorescence. During this period, it was seen that adult beetles even did not like to sit on the older plants having tough texture of the leaves.

The defoliation of Parthenium is of population dependant. Population build-up is also dependant on rains and temperature. After rains in June-July, population build-up starts but long dry spell can reduce the population build-up of the beetle drastically. At Jabalpur, continuous or intermittent rains during June to September resulted high defoliation in large area by the end of August but dry spell of 15 to 20 days during June-July resulted same defoliation by the end of September. Scanty rains or draught conditions during rainy season may cause severe set back in population build-up of beetle hence poor defoliation of Parthenium. Jayanth and Bali (1992) estimated 5-6 generations under filed conditions in Bangalore on the basis of summation of thermal units. Gautam *et al* (2007) found mean maximum population (121 adults/plant) of *Zygogramma bicolorata* during September 2005 followed by 97 adults/plant in May 2005 as against low population during rest of the period. They also observed beetles continuously from January 2005 to June 2006 in Delhi climate.

***Natural enemies of Z. bicolorata:*** Some predators and parasites have been reported which may reduce the survival rate of the beetle. A predatory assassin bug identified as *Cantheoconidea furcellata* Worlf was found to feed on grubs of the *Z. bicolorata* in Madhya Pradesh (Sushilkumar 1997*,* Sushilumar 2005). Both adults and larvae of *C. fercelleta* predate *Z. bicolorata* larvae in the field during July to October but in abundance during September. A single adult predator may devour 6-12 grubs in a day. A tachinid parasitoid *Palexorista* sp. was reported attacking 3rd- and 4th-instar larvae of *Z. bicolorata* from Karnataka in India (Jayanth *et al*. 1996), Pandey *et al*. (2003) recorded a predator *Andrallus Spinidens* on Mexican beetle from Uttranchal, India. Gupta *et al*. (2004) reported three species of predatory bugs namely *Andrallus spinidens* Fab., *C. furcellata* Wolf. and *Sycanus pyrrhomelas* Walker. The former two pentatomid bugs feed exclusively on grubs while latter reduviid predated upon both grubs and adults. They found that a single bug of *A. spinidens* and *C. furcellata* consumed on an average of 23 and 21 grubs, respectively whereas *S. pyrrhomelas* consumed 12 and 7 grubs and adults of *Z. bicolorata*, respectively. First time Sushilkumar (2005) recorded a fungus *Beauveria bassiana,* an entomopathogenic fungus on *Z. bicolorata* killing culture of larvae in the laboratory during rearing. The eggs and grubs of the bioagent were found prayed by many variety of ants and spiders. Garden lizard (*Chamaeleo chamaeleon*), house sparrow (*Passer domesticus)* and common myna (*Acridotheres tristis*) were also reported to feed on grubs and adults of *Z. bicolorata* (Sushilkumar 2005).

**Spread and establishment of Mexican beetle in India**

Field releases of the beetles were initiated in Bangalore in 1984. Although the insect established readily, population build-up was noticed only in 1988 and by 1994, it had spread over 200 000 sq km area in and around Bangaluru from the epicenter (Jayanth and Visalakshy 1994). However, the Controversy emerged over the feeding of beetle on sunflower and imposition of ban by Govt. of India in 1992, hampered the introductory releases of this beetle drastically in newer areas hence its spread and distribution too. By 1992, beetle had spread and established in Karnataka, Tamil Nadu and Andhra Pradesh besides in some parts of Maharashrtra and in Vindhyanagar of district Sidhi, Madhya Pradesh. Efforts made during 1989-1992 by Indian Institute of Horticultural Research, Bangalore for release of beetle in Punjab, Himachal Pradesh and Jammu & Kashmir also yielded good success by the beetle in some of the areas in future.

Further efforts made by Directorate of Weed Science Research Jabalpur, and from ICAR institutes and university from Bangaluru for release of Mexican beetle after lifting of ban on beetle's release (Sushilkumar and Yaduraju 2003, Sushilkumar 2005b), helped in the establishment of beetle in many parts of Madhya Pradesh, Haryana, Delhi, Lower Uttrakhand and western Uttar Pradesh. Initially, it was thought that Mexican beetle will be suitable only in moderate climate and will not be able to establish well in the areas having low and high temperature extremes below and above 15 and 35$^0$C, respectively (Jayanth and Bali 1993b). But recent survey betrayed this assumption as beetle was found to cause large-scale defoliation at Vindhya Nagar (Sushilkumar and Bhan 1997b), Jabalpur of Madhya Pradesh (Sushilkumar 2005b), Rudrapur, Kashipur, Panthnagar, Jaspur, Roorkee, Dehradun, Rishikesh, Haridwar (Uttaranchal), Gajiabad, Bijnor, Saharanpur (Uttar Pradesh) (Sushilkumar 2005a, 2005b). In Punjab, beetle was found well established in Jalandhar, Ropar, Ludhiana and near Wagha border of India and Pakistan. It was surmised that beetle from this root may enter in to Pakistan and will exert biotic pressure on the Parthenium population (Sushilkumar, 2005, 2006a). After this publication, Javaid and Shabbir (2007) reported the occurrence of beele from Lahor which confirmed the entry of *Z. bicolorata* from this route to Pakistan. In Haryana, beetle was found established in some parts of Rohatak, Sonipat, Kaithal and Karnal. The beetle was also recovered in Delhi in 2004 from the released sites after its introduction in 2001 and now it is well established in and around Delhi. These regions in north and central India represent low and high temperature regime thus further strengthen the evidences of potential of Mexican beetle for biological control of Parthenium throughout the country.

From the year 2001 to 2009, the author has sent nucleus culture of beetle to many parts of India ( total culture of about 0.6 million) representing centers of All India Co-ordinated Programme on Weed Control (AICRP-WC), Indian Council of Agricultural Research (ICAR) Institutes, Municipalities, Non-Government Organizations (NGOs) and Krishi Vigyan Kendra (KVKs) by postal service in special developed packing. These introductory releases has also helped to spread this beetle in new areas as there were encouraging reports of sign of establishment in many of the introduced area, however in different intensity of incidence. About two million beetles were released during 2009 in Nagpur district of Maharashtra state alone by Directorate of Weed Science Research in an effort to establish the beetle in the region on the request of Maharashtra Agricultural Department.

In India, *Z. bicolorata* has well established in many parts of Karnataka, Maharashtra, Madhya Pradesh, Bihar, Delhi, Haryana, lower hills of Himachal Pradesh, Punjab, Uttar Pradesh and lower hills of Uttrakhand while it has medium spread and establishment in Andhra Pradesh, Delhi, UP, Orissa, Rajasthan, Tamil Nadu, upper hills of Uttrakhand and Himachal Pradesh. It has low spread and establishment in Assam, Jharkhand, Gujrat, Kerala West Bengal. *Z. bicolorata* has nil to negligible spread in Andman & Nicobar, Arunachal Pradesh, Goa, Meghalaya, Mizoram, Manipur., Sikkim *etc.* In Tamil Nadu and Andhra Pradesh *Z. bicolorata* has been well spread only in western and northern and north and west regions, respectively. In general, the incidence and spread of *Z. bicolorata* was recorded very limited in all the coastal regions besides cold and hot deserts of India.

On the basis of current distribution of *Z. bicolorata* in India, it is suggested that the geographic range of this bioagent can extend to other Parthenium infested areas in coastal region, arid region and north-east states as well as in the Andaman & Nicobar Islands, where the agent is currently in very low states or not known to occur. The spread and establishment of *Z. bicolorata* is likely to extend more in Andhra Pradesh, Bihar, Chhattisgarh, Delhi, Haryana, Himachal Pradesh, Madhya Pradesh, Rajasthan, Uttar Pradesh and Uttrakhand. A more systematic survey is required to delineate the spread and establishment of *Z. bicolorata* in India. Dhileepan and Senaratne (2009) presented a distribution map of Parthenium and *Z. bicolorata* based on a GIS-based distribution and on meta-analysis in South Asia. A Climex model based on the current distribution of *Z. bicolorata* in India suggests that the geographic range of this agent in India and Pakistan can extend to other *P. hysterophorus*-infested areas in the region. The Climex model also suggests that all of Bangladesh and Sri Lanka, and parts of Nepal are climatically suitable for *Z. bicolorata.*

***Mass multiplication of Z. bicolorata* :** Initial attempts of mass multiplication was made by Jayanth (1987) in the laboratory and net house on the Parthenium leaves and pots, respectively. Demand of beetle increased with increasing success of *Z. bicolorata* to release in other areas of India. Sushilkumar (2005) made serious efforts to multiply large number of beetles in the net houses by sowing the seeds of Parthenium and subsequent planting of seedlings after defoliation by the beetle. With these techniques, it was possible to mass rear the beetle in thousands of number. Techniques were also developed to mass rear the beetle in agro-net houses during summer and in poly houses in winter (Sushilkumar 2005, Sushilkumar and Ray 2008). These techniques were found helpful to augment the mass reared beetle with start of rains to increase the population build-up and subsequent control (Sushilkumar 2008).

Murthy *et al. (*2009) has also attempted to mass rear the *Z. bicolorata* on a semi-synthetic diet utilizing parthenium leaf powder *in vitro* conditions at 26±1°C, 65% RH. Significant differences were observed in the biological parameters of the beetle *viz.*, the grub survival (54.3%), pupation (47.2%), fecundity (54.6/day) and total developmental period (37.7 days), when reared on the semi-synthetic diet compared to those when reared on natural parthenium leaves (91.5, 85.5, 74.8 and 29.8, respectively).

**Impact of Mexican beetle on parthenium density, biomas, and seed bank**

Due to heavy population build-up of the beetle in many areas in India, Parthenium was completely defoliated. In such areas, where large-scale defoliation of Parthenium had taken place, the weed density was observed to be reduced in due course. Spectacular success of Parthenium suppression by Mexican beetle was experienced at Bangaluru (south India) during 1990-1992 after its introduction and establishment (Jayanth and Bali 1994). The impact of Mexican beetle on plant height, flower production and stand density was recorded. During 1988, fully-grown plants measured 80 to 200 cm with a mean height of 97.56 cm. Corresponding figures for mean height in 1989 and 1990 were 81.24 and 64 cm, respectively. The flowering period and production of flowers were also severely affected by insect defoliation. Plants in the study area that had produced about 4720 flowers in 20-week flowering period in 1988, produced only 168 flowers during a decreased six-week flowering period in 1989, and in 1990 the flowering period lasted only four weeks with a mean production of just 123 flowers. There was also a decline in the density of weed growth. In 1988, about 40 to 70 plants (mean 48) could be counted per square metre. This number was reduced by 52 per cent in 1989 and a further 63 per cent in 1990. Defoliation of parthenium by *Z. bicolorata* was found to cause up to 98% reduction in flower production and also ensure the growth of vegetation formerly suppressed by this weed (Jayanth and Bali, 1994). Field studies also showed that ploughing of fallow agricultural fields, after defoliation of parthenium by *Z. bicolorata* resulted in renewed weed growth (Jayanth and Vislakshy, 1996). This may be due to the presence of over 2 million viable seeds per km of infested soil as reported by Joshi (1990).

In 1991, about 10,000 adults of *Z. bicolorata* from Bangalore were introduced in the campus of Vindhyanagar National Thermal Power Corporation (VNTPC) in the district Sidhi of Madhya Pradesh (India). Parthenium mean height and density was reduced after beetle's invasion from 1.93 m and 77/$m^2$ to 0.48 m and 15.5/$m^2$, respectively. The flower production and dry weight was reduced up to 93.3 and 67% than the control. This repeated defoliation over a period of four years allowed germination of other native vegetation among which *Cassia tora* was in abundance. This region represents extreme fluctuations in temperature and humidity as in winter, temperature become less than 8°C and in summer it scaled up to 45°C (Sushilkumar and Bhan 1996, 1997b). The success of *Z. bicolorata* under these extremes of temperature clearly indicates its potential in varied climatic conditions of India.

Study carried out at Jabalpur in central India revealed that due to continuous attack of beetle for four years during rainy season, Parthenium population was drastically decreased by more than 80% and the land which used to be dominated only by the Parthenium, had other vegetation among which *C. tora* was most predominant. The most interesting behaviour of beetle in central India was one extra generation during February-March which further helped in early population build-up of the beetle in the same area during rainy season. In such sites, Parthenium flush, which germinated during June-July rains, was killed by the beetle during mid August and new flush of Parthenium germinated in August and early September was nipped in the bud. Drastic reduction in flower production of second and third flush of Parthenium during rainy season is brought about by gregarious feeding by the early larval stages of the insect on the terminal and axillary buds. This feeding does not allow growth of the young plants and they are nipped in the bud.

Mexican beetle was found to defoliate large area of Parthenium in the forest of Rajajee National Park in Uttrakhand state (Goyal and Brahma 2001). Survey by the author also revealed heavy defoliation of Parthenium in this park during 2004. *Z. bicolorata* has also been found to establish in the outer zone of Pench National Park near Seoni in Madhya Pradesh in 2009.

In Australia, Dhileepan *et al*., (2000) and Dhileepan, (2007) also recorded impact of the Mexican beetle in field. They reported 92% defoliation in about 90 days with 27% reduction in plant height and 81% reduction in shoot biomass besides 81 and 73% reduction in flower production and soil seed bank, respectively. Dhileepan (2007, 2009) found significant increase in grass production due to biological control but only in 1 of 4 yr at Mt. Panorama and 2 of 4 yr at Plain Creek. At Mt. Panorama, there was 40% increase in grass biomass in 1997 because of defoliation by *Zygogramma bicolorata* and galling by *Epiblema strenuana*. At Plain Creek, grass biomass increased by 52% in 1998 because of *E. strenuana* and by 45% in 2000 because of combined effects of *E. strenuana* and the summer rust *Puccinia melampodii*. This study provides evidence on the beneficial effects of biological control of parthenium in areas

***Impact of Mexican beetle on Parthenium at biochemical level***

Sushillkumar *et al*.(1997b) studied the effect of defoliation of Parthenium by the beetle and mechanical damage in terms of changes in total phenol (TP) and carbohydrate content. Total phenol and carbohydrate content when quantitated in relation to 30 and 50% of local damage of leaves by beetles revealed that amount of phenols decreased by 4.03 and 4.95 fold at 30 and 50% defoliation of parthenium, respectively. Total phenols and sugars content were gradually decreased corresponding to increase in percentage of defoliation. In case of whole plant damage, analysis showed that TP content decreased steadily with defoliation both by the beetles chewing and by mechanical means.

**Economic benefits of biological control by *Z. bicolorata***

Biological control of Parthenium is especially attractive in wasteland, community land and forests which are sensitive ecosystems important for human health, animals and wildlife. In these areas, only government efforts can do something to control Parthenium because common men and farmers seldom take interest in such situations, as control is not directly beneficial to them. At present, beetle has established firmly in many parts of north, central and south India. Beetle has contributed significantly to suppress Parthenium in large area and helped indigenous species to re-establish, thus saving loss of biodiversity.

Ecological benefit in the form of re-germination of lost vegetation and hence saving of loss of biodiversity has been reported (Jayanth and Vishalakshy, 1996, Sushilkumar 2005). A study made at Jabalpur by Sushilkumar (2006b) to find out the economic benefit by *Z. bicolorata* after release of 6000, 7500, 7500 beetles in the year 2000, 2001 and 2002, respectively. Based on the herbicide cost which would have incurred in the area controlled by beetle, the net economic return by third year was calculated 135% per annum, which increased to 608, 2700, and 12150% per annum for single application of herbicides by 4$^{th}$, 5$^{th}$ and 6$^{th}$ years, respectively. The total benefits by the biological control in six years had been of Rs 62.34 million; 15585% benefit over initial investment. Sushilkumar (2006b) concluded that return would have increased many folds if benefits derived in the form of environmental safety and sustainability is taken into consideration. It has also to be considered that during rainy season, Parthenium germinates in flushes after commencement of rains till the end of rainy season. Hence, at least two applications of herbicides are required to control Parthenium which would further double the cost.

The economic benefit in terms of grass production has also been reported in Australia by Dhileepan (2009). In 1940s, efforts made to control South American weed *Cordia macrostachya* in the Indian Ocean Island Mauritius by biological control yielded economic benefits by 1000 per cent per annum since the introduction of the bioagent (Simmonds 1967). He also cited the example of biological control of *Opuntia* cactus in Nevis in the years (1957-60), which would have cost some 2000 per cent return if controlled by the herbicides. The range of economic returns varied 200-25,000 per cent of the investment. In the present case of biological control of parthenium by *Z. bicolorata*, there was less return during initial years but with the establishment and spread of the bioagent, economic returns increased in subsequent years.

**Prospects of biological control in India**

Past efforts through biological control indicate that Parthenium management through bioagents can not be as simple as in some other successful cases because of high regeneration capacity, large seed production ability, germination ability through out the year and extreme adaptability of Parthenium in wide range of ecosystem. Bhan *et al.* (1997) suggested some future strategies to manage the Parthenium by integrated methods. Sushilkumar and Saraswat (2001) emphasized the need of integrated management with biointensive approach. Some future strategy or Parthenium management may be as follows :

(i) Efforts are required to look into the case of failure of past efforts in establishing of seed feeding weevil *Smicronyx lutulentus*. The establishment of this insect alongwith *Z. bicolorata* may be helpful to manage Parthenium more effectively.

(ii) So far only *Z. bicolorata* has sbeen proved to be a successful bioagent but this bioagent alone is not sufficient to manage Parthenium because of the reason that this beetle is able to make sufficient population build up only during July to September in the area where monsoon rains are received. But, Parthenium is able to germinate throughout the year. The idea of importation and colonization of additional natural enemies, such as the leaf mining moth *Bucculatrix parthenica*, the seed feeding weevil *Smicronyx lutulentus* and the fungal pathogen *Puccinia abrupta* var. *partheniicola*, may complement *Z. bicolorata* for *P. hysterophorus* control throughout India.

(iii) Augmentation of *Z. bicolorata* can be achieved through mass multiplication. More concentrated efforts are needed to mass multiply *Z. bicolorata* throughout the season as suggested by Sushilkumar (2005).

(v) So far, only countable efforts have been made in India to make effective mycoherbicides. More concentrated research in this direction is imperative in the development of mycoherbicides for effective Parthenium management. It has been reported that integration of bioherbicides with reduced rate of herbicides can successfully improve the activity of mycoherbicides towards weed. It has also been suggested that bioherbicides comprised of native pathogens may be more effective than those compared of introduced pathogens because of more readily adaptability.

(vi) The role of marigold should get encouragement in integrated Parthenium management in residential colonies, office premises and farm houses with the help of people's participation. Using marigold, Parthenium suppression can be achieved at one hand while aesthetic value can be maintained on the other hand.

(vii) The competitive and harmless plants like *Cassia sericea* and*, C. tora* should be used in the integrated fashion to manage the weed biologically.

(viii) Safe herbicides can also be integrated with bioagents.

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