DWR/2025/3

Training Manual

Bio agents Based Sustainable Weed Management for Healthy Ecosystem

Edited and Compiled

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Correct Citation: Anokhe A. and Deeksha M. G. (2025). Bio agents Based Sustainable Weed Management for Healthy Ecosystem. Training Manual No. 3, ICAR- Directorate of Weed Research, P. 30.

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Layout & Design:
Sandeep Dhagat

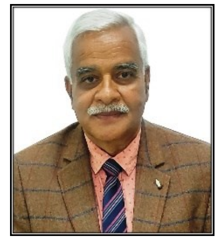
Published by
Director, ICAR- Directorate of Weed Research, Jabalpur- 482004, India

Further Information:
ICAR- Directorate of Weed Research, Jabalpur- 482004
Phone: 0761-2353934, 2353101
e-mail: director.dwr@icar.org.in
website: https://dwr.org.in

ISBN : 978-81-985626-3-0

9 788198 562630

FOREWORD



Weeds continue to challenge sustainable agriculture and ecosystem health by competing with crops for vital resources, degrading habitats, and facilitating the spread of pests and diseases. The reliance on chemical herbicides, although effective in the short term, has raised concerns due to environmental pollution, herbicide resistance, and adverse effects on non-target organisms. In this context, biological control utilizing natural enemies such as insects, pathogens, and microbial agents emerges as an ecologically viable and long-term solution for weed management.

The Training Manual on *"Bioagents-Based Sustainable Weed Management for Healthy Ecosystem*" is a timely and significant contribution by the ICAR–Directorate of Weed Research, Jabalpur. This manual provides a structured and practical framework for understanding and implementing biological weed control strategies. It comprehensively addresses key topics, including the importation and quarantine protocols of bioagents, climate change implications for weed and biocontrol dynamics, and the effective management of both aquatic and terrestrial invasive weeds through classical and augmentative biocontrol techniques.

Notably, the manual highlights the importance of *mass multiplication protocols* for bioagents to ensure their effective field-level deployment, while also exploring the emerging potential of *microbial herbicides* in integrated weed management. The inclusion of a chapter on the *utilization of invasive weeds* further reflects a holistic approach, promoting their conversion into valuable resources such as compost, bioenergy, or animal feed.

This publication serves not only as a training resource but also as a strategic guide for researchers, students, extension personnel, and policymakers working towards sustainable, bio-based weed management systems. I commend the authors and contributors for their dedication in compiling this important manual and trust it will prove instrumental in strengthening capacity-building efforts and advancing weed science research in India and beyond.

(J. S. Mishra)
Director

PREFACE

Weeds pose one of the most persistent threats to agricultural productivity, biodiversity, and the overall health of ecosystems. Invasive weed species not only outcompete native vegetation but also alter ecological processes, affect water regimes, and disrupt food chains. As the limitations and environmental consequences of chemical herbicides become increasingly evident, there is a pressing need to adopt sustainable, ecologically sound approaches for weed management. Biological control, which involves the use of natural enemies such as insects, pathogens, and microbial agents, offers a viable and environmentally benign solution to manage invasive weeds across diverse ecosystems.

The Training Manual on *"Bioagents-Based Sustainable Weed Management for Healthy Ecosystem"* has been developed as a comprehensive training manual under the aegis of ICAR-Directorate of Weed Research, Jabalpur**.** It serves as a guide for researchers, extension personnel, students, and stakeholders engaged in the field of weed science, biological control, and sustainable agriculture. This manual covers a wide range of critical topics. It begins with the *Importation and Quarantine Procedures* essential for the safe introduction of exotic insect agents for weed biocontrol. It addresses the evolving challenges posed by *climate change in weed management* and its implications for the performance and adaptability of biocontrol agents. Dedicated chapters discuss the *biocontrol of aquatic and terrestrial weeds*, focusing on successful case studies, agents used, and ecosystem restoration impacts. Practical aspects are highlighted in the section on *Principles and Techniques for Mass Multiplication of Weed Bioagents*, offering hands-on protocols for large-scale implementation. Furthermore, the manual explores the promising area of *microbial herbicides*, underscoring their potential to complement classical biocontrol strategies. A forward-looking chapter on the *Utilization of Invasive Weeds* discusses innovative approaches to repurpose weed biomass for beneficial applications, promoting circular bioeconomy concepts.

Through this manual, the ICAR–Directorate of Weed Research reinforces its commitment to fostering nature-based, sustainable weed management strategies. We hope this publication empowers participants to effectively implement and advocate bioagent-based weed management approaches that align with the goals of environmental protection, biodiversity conservation, and resilient agroecosystems.

Authors

CONTENTS

IMPORTATION AND QUARANTINE PROCEDURES OF INSECT AGENTS FOR BIOLOGICAL CONTROL OF WEEDS

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Introduction

The importation of exotic natural enemies for managing insect pests and weeds is commonly referred to as classical biological control. Table 1 presents the list of arthropods and other organisms introduced into India for the biological control of weeds. All these biological control agents were initially quarantined at the quarantine laboratory of the Commonwealth Institute of Biological Control (CIBC), Indian Station, and later at the ICAR–National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. Biological control agents that successfully passed host-specificity testing were subsequently handed over to the importing agencies for further research and field release.

Pre-introduction studies

Before initiating a biological control program, it is essential to assess whether the target weed has any known beneficial uses and to address potential conflicts of interest. All stakeholders must reach a consensus that the plant in question is indeed a problematic weed. Unlike chemical or cultural control methods, biological control cannot be confined to a specific area, as the introduced living organisms can spread beyond the initial release site.

In weed biological control, host-specific organisms are typically utilized. These species, having co-evolved with their host plants, are most likely to be found in the weed's center of origin. Therefore, explorations for suitable control agents generally begin in that region.

Surveys are conducted in the weed's native country to identify host-specific natural enemies. Simultaneously, surveys in the country requiring control are carried out to determine whether any of these natural enemies are already present, thereby avoiding the unnecessary and costly reintroduction of species.

Preliminary host-range studies are performed in the weed's native country to assess the specificity of potential natural enemies. These studies are followed by detailed research on their life cycle, feeding behavior, and other biological traits to identify the most suitable candidates for importation.

Introduction and host-specificity tests under quarantine conditions

The import of weed-feeding insects must be authorized through special permits issued in Form PQ-13 by the Plant Protection Advisor to the Government of India (Fig. 1). These imports must be transported by air through designated airports, including New Delhi, Chennai, Kolkata, Mumbai, Amritsar, and Bengaluru, or via other officially notified points of entry. Each consignment of beneficial insects must be accompanied by a phytosanitary certificate issued by the National Plant Protection Organisation (NPPO) of the country of origin. This certificate must include additional declarations confirming that the insects are free from specified parasites, parasitoids, and hyperparasites. Upon arrival in India, the consignment is subjected to post-entry quarantine procedures as prescribed by the Plant Protection Advisor. The ICAR–National Bureau of Agricultural Insect Resources (ICAR–NBAIR) possesses the necessary quarantine facilities to conduct all mandatory studies required for the safe evaluation and release of imported weed biocontrol agents.

Introduction and quarantine multiplication

Natural enemies are typically received via air-freight shipments and are unpacked in a quarantine laboratory to prevent the unintentional introduction of parasites, predators, pathogens, or other harmful organisms. Breeding cultures are then established and maintained under quarantine conditions until the required permits for field release are obtained.

Host-specificity studies

A critical aspect of any biological control program for weeds is ensuring that the biocontrol agent can be introduced without posing a threat to beneficial or desirable plants. To confirm this, host-specificity tests are conducted under quarantine conditions to ensure that the weed biocontrol agent will not harm non-target plants after release. It is essential to finalize the list of test plants early in the biocontrol program to facilitate efficient research planning and to avoid delays caused by the late inclusion of additional test plants after the screening process is completed.

No-choice test

In biological control programs, it is not feasible to test every plant species present in the target country. Therefore, a strategic selection of test plant species is essential. The primary objective is to assess the potential host range of the candidate biological control agent. To accomplish this, a diverse set of representative plant species from various taxonomic groups is selected for testing. These tests are conducted with multiple replications and involve a sufficiently large number of individuals to ensure reliable and statistically sound results. The data obtained from these preliminary tests serve as a basis for designing more refined host-specificity experiments.

Free choice/ multiple-choice tests

In multiple-choice tests, insects are simultaneously exposed to several plant species, including their known host plant. The results of these tests are considered valid only if normal feeding occurs on the host plant, confirming that the insects are exhibiting natural behavior. These tests are typically conducted using adults or larvae capable of moving freely between the different plants, allowing researchers to accurately assess feeding preferences and host specificity.

Oviposition tests

Oviposition tests are conducted using adult insects to determine their preferred sites for egg-laying and where their offspring will subsequently feed. These tests are particularly important for insect species in which adults lay eggs directly on the host plant and the larvae have limited mobility, preventing them from relocating to other plants. Such species include leaf miners, gall formers, borers, and seed feeders.

Test plants should be monitored regularly, with detailed observations recorded, including the extent of plant material consumed and the survival status of the test arthropods. Special emphasis should be placed on determining whether the insect can complete its life cycle on the test plant. If a plant supports normal feeding and development under field conditions, the candidate biological control agent is typically rejected. Ideally, testing should continue until all insects have died or the test plant is completely consumed.

Wherever possible, host-specificity tests should be conducted using whole plants rather than cut plant parts to avoid chemical and physical changes after cutting, which may influence insect behavior and test results. Whole plants also enable the extension of tests into developmental studies, allowing researchers to assess whether the test plants can support full insect development through to the production of fertile adults.

Despite all precautions, false-positive results may sometimes occur- for example, a plant may be accepted under laboratory conditions but not under natural field conditions. When such discrepancies are suspected, more accurate conclusions can be drawn by conducting tests under semi-natural conditions. These include placing potted test plants outdoors among natural stands of the weed or establishing experimental plots with a mix of the host and test plants, which are then artificially infested with the candidate biocontrol agent. When testing is conducted in the target country, these semi-natural tests should be performed within quarantine greenhouses using potted plants.

Although semi-natural condition tests are the most reliable, they are often resource-intensive, time-consuming, and logistically challenging. To streamline the process, a tiered approach is followed, beginning with simple no-choice feeding tests using excised plant parts. If any damage is observed, the affected test plants are then assessed in more complex multiple-choice tests using whole plants. If damage persists, these plants are further tested under semi-natural conditions.

This three-tiered testing strategy minimizes the risk of incorrectly rejecting a safe biocontrol agent or approving a potentially harmful one. Only candidates that present possible risks are advanced to the more detailed and costly testing phases.

If the series of tests indicates that a candidate biocontrol agent is not strictly monophagous (i.e., it feeds on more than one plant species), a comprehensive risk assessment must be undertaken. A final decision must be made regarding whether the level of risk to non-target plants is acceptable for release.

Procedures for carrying out host range screening tests

In the past, host plant selection for host-specificity testing was often done arbitrarily. However, current guidelines recommend a more systematic and scientifically grounded approach. Host plants are now selected based on their taxonomic, chemical, and morphological similarities to the target weed. This structured approach is known as the *centrifugal testing scheme*, in which testing begins with plant species closely related to the target weed and gradually expands to include more distantly related species.

Guidelines for Selecting Test Plants in Host-Specificity Screening (Centrifugal Testing Scheme)

When designing host-specificity tests for a candidate biological control agent, a tiered and systematic approach should be adopted to ensure a comprehensive risk assessment. The selection of test plant species should represent a broad spectrum of taxonomic, chemical, ecological, and economic relationships to the target weed. The following categories should be included:

***Target species and its varieties*:**

Begin testing with the target weed species itself, including known varieties or subspecies present in the intended release region.

Species from the same genus:

Include other species within the same genus to evaluate host specificity among closely related taxa.

Species from related genera within the same tribe:

Add plant species from different genera that belong to the same botanical tribe as the target weed.

Species from genera in closely related tribes:

Expand testing to include species from genera in tribes that are taxonomically related to the tribe of the target weed.

Species from closely related families:

Include representative species from families related to the target weed's family, providing broader phylogenetic coverage.

Chemically similar species:

If available, incorporate plant species with chemical profiles similar to the target weed, as shared chemistry may influence insect host selection.

Economically or ecologically important plants:

Include crops, ornamentals, and native or endangered species of economic or ecological significance in the region of release to assess potential non-target risks.

Recorded host plants of the candidate agent:

Add plant species previously reported as hosts of the candidate biological control agent to confirm or reassess historical host associations.

Host plants of related insect species:

Include species known to be hosts of other insect species within the same genus as the candidate agent to evaluate possible host-range overlap.

Field Releases, Monitoring, and Evaluation

Field Release Permits

Once host-specificity testing is completed, the results are submitted to the Plant Protection Adviser to the Government of India. A panel of scientific experts reviews the data to assess the safety and specificity of the candidate agent. Based on their evaluation, the expert committee may recommend the issuance of permits for limited field trials, ensuring that environmental safety and efficacy are validated before widespread release.

Mass multiplication and initial field releases

Before the biological control agent is released into the field, mass rearing techniques are developed and standardized. Initial releases are conducted in undisturbed locations, ideally in areas where the target weed is present year-round, to support the survival and establishment of the agent.

Monitoring and Evaluation

Post-release, the following parameters are closely monitored to assess the performance and impact of the introduced agent:

- Establishment of the agent in the release area
- Population buildup over time
- Dispersal from the release site to surrounding areas
- Adaptability to local climatic conditions
- Interaction with indigenous natural enemies (e.g., predators or parasitoids)

The evaluation of these factors helps assess the potential success of the biological control program and determine whether the importation of additional natural enemies is necessary for effective weed management.

Table 1. Arthropods and other organisms introduced into India for the biological control of weeds

No.	Year	Name of Weed	Biological Control Agent	Country of Origin
Terrestrial Weeds				
1	1795	*Opuntia vulgaris*	*Dactylopius ceylonicus*	Brazil
2	—	*O. vulgaris*	*D. confusus*	South America
3	1925	*Opuntia dillenii*	*D. opuntiae*	USA / Australia / Sri Lanka
4	1925	*Opuntia elatior*	*D. opuntiae*	USA / Sri Lanka / Australia
5	1921	*Lantana camara*	*Uroplata lantanae*	Mexico / Hawaii
6	1941	*L. camara*	*Teleonemia scrupulosa*	Mexico / Fiji / Australia
7	1972	*L. camara*	*Crocothemis girardi*	Brazil / Hawaii / Australia
8	1972	*L. camara*	*Octotoma scabripennis*	Mexico
9	1975	*Chromolaena odorata*	*Apion brunneonigrum*	Trinidad
10	1981	*C. odorata*	*A. brunneonigrum*	Trinidad
11	1981	*C. odorata*	*Pareuchaetes pseudoinsulata*	Trinidad
12	1984	*C. odorata*	*P. pseudoinsulata*	Sri Lanka
13	1986	*C. odorata*	*Mescinia* sp.	Trinidad
14	1963	*Eupatorium adenophorum*	*Procecidochares utilis*	Mexico / Hawaii / Australia / New Zealand
15	1983	*Parthenium hysterophorus*	*Smicronyx lutulentus*	Mexico
16	1983	*P. hysterophorus*	*Zygogramma bicolorata*	Mexico
17	1983	*P. hysterophorus*	*Epiblema strenuana*	Mexico
18	1982	*Orobanche spp.*	*Phytomyza orobanchiae*	Yugoslavia
19	2018 2019 2025	*P. hysterophorus*	*Smicronyx lutulentus*	Australia
Aquatic Weeds				
20	1865	*Salvinia molesta*	*Osteochromis gourami* (fish)	Mauritius
21	1916	*S. molesta*	*O. gourami* (fish)	Java
22	1953	*S. molesta*	*Tilapia mossambica* (fish)	Africa
23	1959	*S. molesta*	*Hypophthalmichthys molitrix* (fish)	China
24	1959	*S. molesta*	*Ctenopharyngodon idella* (fish)	China / Hong Kong
25	1962	*S. molesta*	*C. idella* (fish)	China
26	1974	*S. molesta*	*Samea acutalis*	Trinidad
27	1982	*S. molesta*	*Cyrtobagous salvinae*	Brazil / Australia
28	1982	*Eichhornia crassipes*	*Orthogalumna terebrantis*	Argentina / USA
29	1983	*E. crassipes*	*Neochetina bruchi*	Argentina / USA
30	1983	*E. crassipes*	*Neochetina eichhorniae*	Argentina / USA

Case study of host-specificity tests undertaken for the biological control agent, *Zygogramma bicolorata* against the target weed *Parthenium hysterophorus* in the year 1983 by Jayanth and Nagarkatti (1987c).

A. No-Choice Tests: Tests were conducted using adult beetles and larvae to evaluate host specificity under restricted conditions. Number of plant species tested: 40; Number of plant families represented: 22

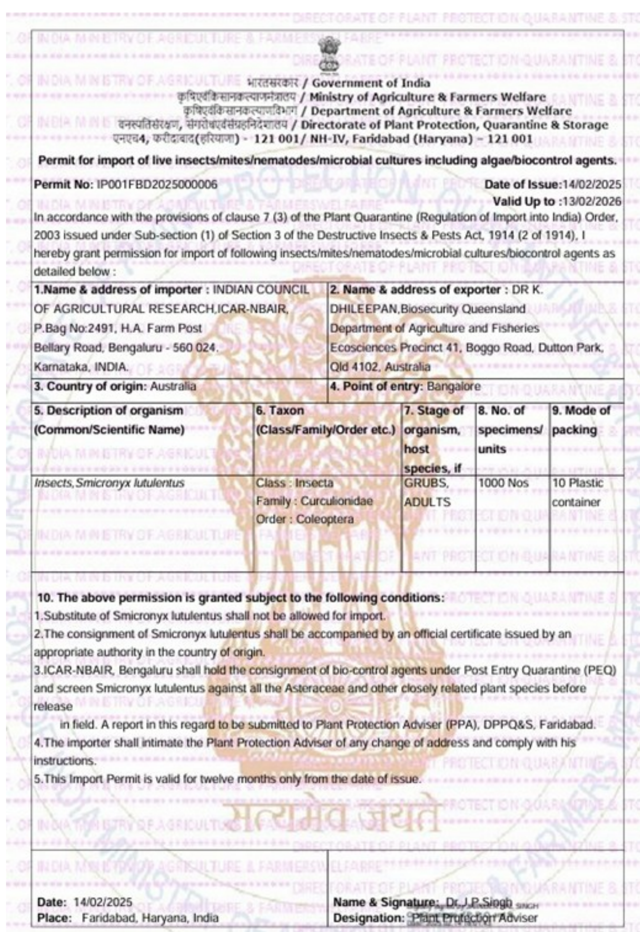
Results: No feeding, oviposition, or larval development occurred on 37 species. Slight feeding (nibbling) observed on 2 species, *Jasminum grandiflorum*: Beetles survived for 34 days, fed for 10 days. *Guizotia abyssinica*: Beetles survived for 18 days, fed for 6 days.

B. Multiple-Choice Tests: Tests allowed beetles to choose among multiple plant species.

Result: Feeding, egg laying, and larval development were observed only on *Parthenium hysterophorus*. No significant activity was recorded on any other plant species.

Conclusion: Based on both no-choice and multiple-choice test results, *Zygogramma bicolorata* showed high host specificity for *Parthenium hysterophorus*.

Recommendation: The species was approved for field release in India as a safe biological control agent.



भारतसरकार / Government of India
कृषिएवंकिसानकल्याणमंत्रालय / Ministry of Agriculture & Farmers Welfare
कृषिएवंकिसानकल्याणविभाग / Department of Agriculture & Farmers Welfare
वनस्पतिसंरक्षण, संगरोधएवंसंग्रहनिदेशालय / Directorate of Plant Protection, Quarantine & Storage
एनएच4, फरीदाबाद(हरियाणा) - 121 001/ NH-IV, Faridabad (Haryana) - 121 001

Permit for import of live insects/mites/nematodes/microbial cultures including algae/biocontrol agents.

Permit No: IP001FBD2025000006 **Date of Issue:** 14/02/2025
Valid Up to : 13/02/2026

In accordance with the provisions of clause 7 (3) of the Plant Quarantine (Regulation of Import into India) Order, 2003 issued under Sub-section (1) of Section 3 of the Destructive Insects & Pests Act, 1914 (2 of 1914), I hereby grant permission for import of following insects/mites/nematodes/microbial cultures/biocontrol agents as detailed below :

1.Name & address of importer : INDIAN COUNCIL OF AGRICULTURAL RESEARCH,ICAR-NBAIR, P.Bag No:2491, H.A. Farm Post Bellary Road, Bengaluru - 560 024, Karnataka, INDIA.	**2. Name & address of exporter :** DR K. DHILEEPAN,Biosecurity Queensland Department of Agriculture and Fisheries Ecosciences Precinct 41, Boggo Road, Dutton Park, Qld 4102, Australia
3. Country of origin: Australia	**4. Point of entry:** Bangalore

5. Description of organism (Common/Scientific Name)	6. Taxon (Class/Family/Order etc.)	7. Stage of organism, host species, if	8. No. of specimens/ units	9. Mode of packing
Insects,Smicronyx lutulentus	Class : Insecta Family : Curculionidae Order : Coleoptera	GRUBS, ADULTS	1000 Nos	10 Plastic container

10. The above permission is granted subject to the following conditions:
1.Substitute of Smicronyx lutulentus shall not be allowed for import.
2.The consignment of Smicronyx lutulentus shall be accompanied by an official certificate issued by an appropriate authority in the country of origin.
3.ICAR-NBAIR, Bengaluru shall hold the consignment of bio-control agents under Post Entry Quarantine (PEQ) and screen Smicronyx lutulentus against all the Asteraceae and other closely related plant species before release in field. A report in this regard to be submitted to Plant Protection Adviser (PPA), DPPQ&S, Faridabad.
4.The importer shall intimate the Plant Protection Adviser of any change of address and comply with his instructions.
5.This Import Permit is valid for twelve months only from the date of issue.

सत्यमेव जयते

Date: 14/02/2025
Place: Faridabad, Haryana, India

Name & Signature: Dr.J.P.Singh
Designation: Plant Protection Adviser

Figure 1. Copy of the import permit obtained for introduction of *Smicronyx lutulentus* from Australia

Reference

Jayanth KP and Nagarkatti S. 1987. Investigations on the host specificity and damage potential of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) introduced into India for the biological control of *Parthenium hysterophorus*. *Entomon* 12: 141–145.

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Quarantine is the gatekeeper of environmental safety in biological control.

CLIMATE CHANGE AND WEED MANAGEMENT: CHALLENGES FOR BIOCONTROL AGENTS

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Introduction

Our planet crossed the critical milestone 1.5º C global average temperature rise in 2024, much ahead of its anticipated time, as compared to the preindustrial average, several irreversible and inevitable changes to our world is destined (Bevacqua et al., 2025). Further atmospheric CO_2 level has increased from 280 ppm in pre-industrial times to 422 ppm in 2025 leading to the warmest decade in last 150 years. Also rising temperatures, erratic precipitation regimes, pollution, etc, are leading to ecological and health impacts. Among all the exacerbating environmental problems, the rise of the invasive plant and weed species, and their impact on our wellbeing is a matter of increasing concern.

So today climate change and biological invasions are two of the most significant threat to our global ecosystems with compounding an exacerbating impact on each other causing serious challenge to the phenology, distribution, diversity and composition of the species. Most of the invasive plant species respond positively to climate change. However, the effects of climate change on exotic plants and their combined impacts is scantily understood. In several cases, climate change may create supplementary and complementary prospects for invasion or generate conditions unsuitable for some invasive species (Merow et al., 2017). Accordingly, the scale of ecological and economic impacts of invasive species may intensify, decline, or remain unchanged. Even though the level of this ambiguity of these environmental changes is high and developing management strategies may appear intimidating, because effects will vary regionally with climate and species traits.

Climate change- impact on species interactions

Human mediated habitat degradation is a boon for invasive plant species and invasion, in turn, can drive massive land transformations. For example, an invasive tree species can convert grassland into forest. This may lead to invasion meltdown, which may cause an acceleration in the number of invasive species and impacts (Figure 1). Climate change may create novel opportunities for species with possibilities of invasive plant benefitting from warmer temperatures and expanded ranges. While native species based on their resilience may exhibit new adaptive responses to the changing climatic conditions or cease to exist. These interactions may have far reaching consequences on ecosystem functioning and services.

The combination of climate change and invasive species cause serious ecological changes including hydrological modifications leading to drought in many equatorial and tropical parts of the world, changes in water chemistry, altered biogeochemical processes, altered dispersal patterns, longer growing seasons in mid and high latitudes, shifts in species' ranges towards the poles and higher altitudes to compensate for increasing temperatures, often leading to decline of some species due to physiological intolerance to new environment, altered food webs, and changes in the physiology and reproductive cycles of plants and animals.

Pollinators are vital component of our ecosystems and provide many ecological and economic services essential for agriculture and maintenance of life on this planet. Species richness of native plant species declined linearly with invasive weed while the abundance and species richness of the native plant dependent taxa like butterflies and bees decreased non-linearly with increasing weed infestation. Thus, the rise in invasive plants impacts food webs impacting higher trophic levels across all habitats.

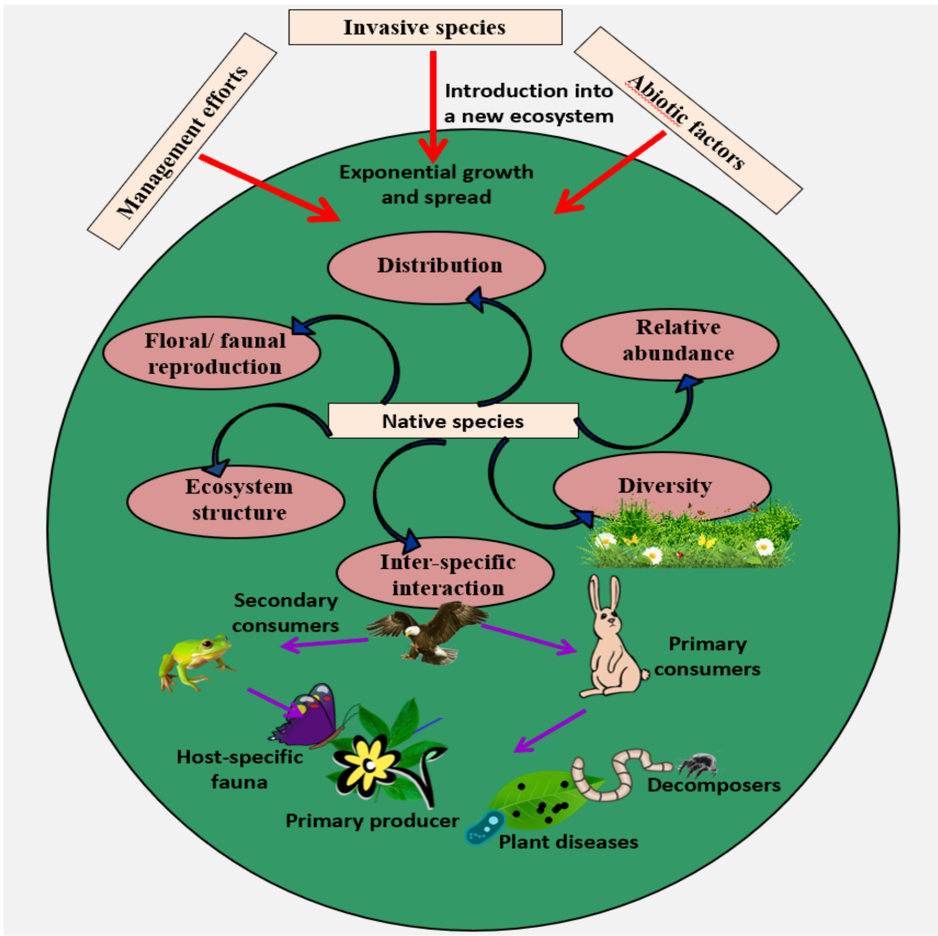

Figure 1. Overall impact of non-native invasive weed on native ecosystem functioning

Climate change- impact on biocontrol of invasive weeds

Evaluation of how individual species respond in an ecosystem and their combined interactions and impact on ecosystems is challenging. Predicting the consequences of climate change on these interactions is difficult not only at species level but also at ecosystem level. Elevated CO_2 and temperature, together with phytopathogenic infection, can significantly alter plant biochemistry and hence plant defense responses. This can have considerable impact on insect fecundity, feeding rates, survival, population density, and dispersal. There is further a possibility of compensatory feeding, increased fecundity and hence population size and distribution. Hence it is essential to establish studies on new biocontrol agents keeping climate variability and climate change in mind, so the information derived from them can be successfully used in future biocontrol programs.

What should be our role as an educated individual:

- Reduce our energy use, shift to renewable energy when possible

- Plant more native trees around us especially in the urban gardens
- Refrain from buying non-native plants and animals
- Repair, reuse, reduce and recycle

Conclusion

As the climate continue to evolve, biological invasions and climate change may act parallelly and amplify each other's effect, which makes it even more imperative to study both phenomena collectively to devise a better tactic to alleviate their effects. In this age of rising globalization, rapid climate and land-use changes, and some mandatory changes in policies is warranted to regulate international and domestic trade, prevent accidental introductions, and manage existent invasions in the country. It is of uttermost importance to promote community awareness through creating educational resources, social media, citizen science programs and capacity building, scientific research, knowledge sharing, and collaboration are the ways for bringing down future invasions by non-native species and mitigating damages from the existing invasions. Lastly it is imperative to further promote related research and use those findings to implement adaptive strategies to mitigate the impacts of invasive plants on native plant communities and dependent taxa. By doing so, we can strive to preserve and restore ecological balance in the face of ongoing environmental change.

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As climates shift, so must our strategies for biological control – flexibility and foresight are key.

BIOCONTROL MANAGEMENT OF AQUATIC WEEDS

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Biological control of aquatic weeds involves the use of natural enemies such as insects, fish, fungi, and bacteria to suppress invasive weed populations in water bodies. This eco-friendly approach reduces the reliance on chemical herbicides, minimizing environmental damage and preserving aquatic biodiversity. Notable biocontrol agents include the weevils *Neochetina eichhorniae* and *Neochetina bruchi*, which effectively control water hyacinth (*Eichhornia crassipes*) by feeding on its leaves and stems, weakening the plant over time. Similarly, the salvinia weevil (*Cyrtobagous salviniae*) is widely used to manage *Salvinia molesta*. These biological agents establish self-sustaining populations, providing long-term weed suppression without continuous human intervention. However, their effectiveness depends on environmental conditions and proper implementation strategies to ensure successful weed management.

Biological Control of Water Hyacinth (*Eichhornia crassipes*)

Water hyacinth (*Eichhornia crassipes*) is one of the most invasive aquatic weeds, causing severe ecological and economic damage. It rapidly spreads over water bodies, blocking sunlight, depleting oxygen levels, and disrupting aquatic ecosystems. It also hampers irrigation, navigation, and fisheries. Due to its fast growth and resilience, biological control has proven to be an effective and sustainable management strategy.

Biological Control Agents

Several insect herbivores and pathogens have been used to control *Eichhornia crassipes*:

1. **Insect Herbivores**
   - *Neochetina eichhorniae* (Water hyacinth weevil): The larvae feed inside the leaf petioles, weakening the plant and reducing its buoyancy.
   - *Neochetina bruchi* (Another water hyacinth weevil): Similar to *N. eichhorniae*, it causes extensive feeding damage, leading to plant decay.
   - *Niphograpta albiguttalis* (Water hyacinth moth): Its larvae bore into the stems, causing wilting and collapse of the plant.
2. **Pathogens**
   - *Cercospora rodmanii* (Fungal pathogen): Causes leaf blight, reducing the plant's ability to photosynthesize.
   - *Myrothecium roridum* (Leaf-spot fungus): Infects leaves, leading to necrosis and plant death.

Effectiveness and Challenges

The introduction of *Neochetina spp.* has successfully reduced water hyacinth populations in several countries, including India. However, biological control alone may not provide complete eradication due to factors such as climate conditions and water nutrient levels that favor regrowth.

For effective long-term management, biological control is often integrated with mechanical removal and water management practices. Ongoing research aims to enhance the efficiency of biocontrol agents and develop sustainable strategies to control water hyacinth infestations in affected water bodies.

Biological Control of *Salvinia molesta*

Salvinia molesta, commonly known as Giant Salvinia, is a free-floating aquatic fern that forms dense mats on water surfaces, blocking sunlight, reducing oxygen levels, and disrupting aquatic ecosystems. It affects fisheries, irrigation, and water transport, making it a serious invasive species in many tropical and subtropical regions. Due to its rapid growth and resistance to mechanical and chemical control, biological control has proven to be the most effective and sustainable management strategy.

Biological Control Agents

1. **Insect Herbivores**
    - *Cyrtobagous salviniae* (Salvinia weevil):
        - The adults feed on leaves, while the larvae tunnel into rhizomes, causing damage that leads to plant collapse.
        - This weevil has been highly successful in reducing *Salvinia molesta* infestations in many countries, including India and Australia.
2. **Pathogens**
    - *Myrothecium roridum* (Leaf-spot fungus):
        - Causes leaf necrosis, reducing the plant's ability to photosynthesize and spread.
    - *Cercospora salviniae* (Fungal pathogen):
        - Infects leaves, leading to wilting and decomposition of the plant.

Effectiveness and Challenges

The introduction of *Cyrtobagous salviniae* has led to significant reductions in *Salvinia molesta* populations, sometimes achieving nearly 100% control within 1–2 years. However, factors such as water temperature, nutrient availability, and plant density can influence the effectiveness of biological control.
For long-term management, biological control is often combined with manual removal and habitat restoration. Continued research focuses on enhancing the efficiency of biocontrol agents and ensuring sustainable suppression of *Salvinia molesta* in affected water bodies.

List of Invasive Aquatic Weeds in India

Invasive aquatic weeds pose a significant threat to India's water bodies, affecting biodiversity, fisheries, irrigation, and overall ecosystem balance. Below is a list of major invasive aquatic weeds found in India:

1. Floating Aquatic Weeds

- *Eichhornia crassipes* (Water hyacinth)
- *Pistia stratiotes* (Water lettuce)
- *Salvinia molesta* (Giant salvinia)
- *Azolla pinnata* (Water fern)

2. Submerged Aquatic Weeds

- *Hydrilla verticillata* (Hydrilla)
- *Myriophyllum spicatum* (Eurasian watermilfoil)
- *Ceratophyllum demersum* (Coontail)
- *Lagarosiphon major* (African elodea)

3. Emergent Aquatic Weeds

- *Typha angustifolia* (Narrow-leaved cattail)
- *Ipomoea aquatica* (Water spinach)
- *Alternanthera philoxeroides* (Alligator weed)
- *Sagittaria sagittifolia* (Arrowhead)

4. Rooted Floating-Leaved Weeds

- *Nymphaea spp.* (Water lily)
- *Nelumbo nucifera* (Sacred lotus)
- *Trapa natans* (Water chestnut)

Impact of Invasive Aquatic Weeds

- **Ecosystem Disruption** – Block sunlight, deplete oxygen, and harm aquatic biodiversity.
- **Agricultural Losses** – Choke irrigation channels and reduce water availability for crops.
- **Fisheries Impact** – Reduce fish populations by altering water quality and habitat.
- **Health Hazards** – Act as breeding grounds for mosquitoes, increasing disease risk.

Control Measures

- **Biological Control** (e.g., *Neochetina eichhorniae* weevils for *Eichhornia crassipes*).
- **Mechanical Removal** (manual or machine-based weed removal).
- **Chemical Control** (herbicide applications where necessary).
- **Integrated Weed Management** (combination of biological, mechanical, and chemical methods).

Water is life, but aquatic weeds can suffocate that life unless we work with nature to restore order.

BIOLOGICAL CONTROL OF TERRESTRIAL WEEDS

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"Biological weed management plays a crucial role, especially in organic and natural farming practices. It not only protects the environment but also serves as an effective long-term solution for sustainable agriculture."

Impact of Weeds on Agricultural Yield

Weeds contribute to approximately one-third of the total agricultural losses (DWR, 2015).
Studies report that weeds cause:

- **5% yield loss** in commercial agriculture,
- **10% loss** in semi-commercial agriculture,
- **20% loss** in subsistence farming, and
- **37–79% loss** in dryland farming (Choudhary & Singh, 2015).

An estimated minimum yield loss of 10% translates to approximately 25 million tons of grain, valued at 13 billion US dollars.

Weed Diversity and Impact in India

In India, **826 weed species** from **61 plant families** are responsible for significant yield losses. Among them:

- **80 species** are categorized as **very serious weeds**, and
- **198 species** are classified as **serious weeds** (Choudhary & Singh, 2015).

Additionally, **62 species** have been identified as **invasive plants** (Reddy, 2008), posing a severe threat to native biodiversity and agricultural productivity.

Biological Method of Weed Management

The biological method of weed management is an eco-friendly and sustainable approach that utilizes natural organisms to control weed populations. This method involves the application of insects, pathogens, parasites, and other biological agents to suppress the growth and spread of weeds.
The primary objective of this approach is to reduce weed infestations to a level where they no longer pose a significant threat to crop yield and quality, ensuring a balanced and healthy agricultural ecosystem.

Key Components of Biological Weed Management

The key components of this technique include:

- **Insects** – Specific insects that feed on targeted weeds, reducing their spread.
- **Pathogens** – Fungi, bacteria, or viruses that infect and weaken weeds.
- **Competition** – Cultivating beneficial plants to suppress weed growth naturally.

Invasive Alien Species (IAS)

Invasive alien species are non-native plants, animals, or microorganisms introduced into an ecosystem, where they rapidly spread and cause harm to native biodiversity, agriculture, and the environment. These species often outcompete native organisms, disrupt ecosystems, and lead to economic losses.

Characteristics of IAS

- Rapid reproduction and high adaptability.
- Lack of natural predators in the new environment.
- Aggressive competition with native species for resources.

Examples of Invasive Alien Species

- **Plants:** *Lantana camara*, *Parthenium hysterophorus*, *Eichhornia crassipes* (Water hyacinth).

Impacts of IAS

- Loss of native biodiversity.
- Reduction in agricultural productivity.
- Disruption of ecosystem balance.
- Economic losses due to increased management costs.

Control Measures

- **Biological control** (introducing natural enemies).
- **Mechanical removal** (manual weeding or trapping).
- **Chemical control** (use of herbicides or pesticides).
- **Legislation & monitoring** (strict regulations on species introduction).

Managing invasive alien species is crucial for protecting native biodiversity and ensuring ecosystem sustainability.

List of Terrestrial Invasive Weeds in India

Invasive weeds pose a significant threat to India's agriculture, biodiversity, and ecosystems. Below is a list of major terrestrial invasive weeds found in India:

1. Asteraceae (Sunflower Family)

- *Parthenium hysterophorus* (Congress grass)
- *Chromolaena odorata* (Siam weed)
- *Ageratina adenophora* (Crofton weed)
- *Mikania micrantha* (Mile-a-minute weed)
- *Bidens pilosa* (Black-jack)

2. Verbenaceae (Verbena Family)

- *Lantana camara* (Lantana)
- *Stachytarpheta indica* (Indian snakeweed)

3. Fabaceae (Legume Family)

- *Prosopis juliflora* (Mesquite)
- *Leucaena leucocephala* (Subabul)
- *Mimosa diplotricha* (Giant sensitive plant)

4. Poaceae (Grass Family)
- *Imperata cylindrica* (Cogon grass)
- *Pennisetum polystachion* (Mission grass)
- *Saccharum spontaneum* (Wild sugarcane)

5. Euphorbiaceae (Spurge Family)
- *Euphorbia geniculata* (Wild poinsettia)
- *Euphorbia hirta* (Asthma plant)

6. Solanaceae (Nightshade Family)
- *Datura stramonium* (Jimsonweed)
- *Solanum viarum* (Tropical soda apple)

7. Convolvulaceae (Morning Glory Family)
- *Ipomoea carnea* (Pink morning glory)
- *Ipomoea purpurea* (Common morning glory)

8. Amaranthaceae (Amaranth Family)
- *Alternanthera philoxeroides* (Alligator weed)
- *Amaranthus spinosus* (Spiny amaranth)

Biological Control of *Lantana camara*

Lantana camara is an invasive shrub that has spread widely across tropical and subtropical regions, causing significant ecological and economic damage. It competes with native vegetation, reduces biodiversity, and affects agricultural productivity. Due to its aggressive nature and resistance to conventional control methods, biological control has emerged as a sustainable and eco-friendly approach to managing *Lantana camara*.

Biological Control Agents

Several biological control agents, including insects and pathogens, have been introduced in different regions to suppress *Lantana camara* populations.

1. **Insect Herbivores**
    - *Teleonemia scrupulosa* (Lantana lace bug): Feeds on lantana leaves, causing defoliation and weakening the plant.
    - *Uroplata girardi* (Leaf-mining beetle): Damages leaves by tunneling through them, reducing photosynthesis.
    - *Ophiomyia lantanae* (Seed-feeding fly): Targets lantana seeds, reducing its reproductive capacity.
    - *Octotoma scabripennis* (Leaf-feeding beetle): Causes severe leaf damage, limiting the plant's growth.
2. **Fungal Pathogens**

- *Puccinia lantanae* (Rust fungus): Infects lantana leaves, leading to premature defoliation and stunted growth.

Effectiveness and Challenges

Biological control of *Lantana camara* has shown variable success. In some regions, biocontrol agents have significantly suppressed lantana populations, but complete eradication is difficult. Factors like climatic conditions, lantana's genetic variations, and the adaptability of control agents influence effectiveness.
For effective management, biological control is often integrated with other methods like mechanical removal and chemical control. Research is ongoing to identify more efficient biological control agents to enhance the suppression of *Lantana camara* and restore affected ecosystems.

Biological Control of Siam Weed (*Chromolaena odorata*)

Siam weed (*Chromolaena odorata*) is an aggressive invasive species that spreads rapidly, forming dense thickets that suppress native vegetation, hinder agricultural productivity, and pose a fire hazard. Due to its rapid growth and ability to regenerate after mechanical removal, biological control has been a preferred sustainable management strategy.

Biological Control Agents

Several natural enemies have been introduced in different regions to suppress *Chromolaena odorata* populations effectively:

1. **Insect Herbivores**
   - *Pareuchaetes pseudoinsulata* (Defoliating moth): Its larvae feed on Siam weed leaves, causing significant defoliation and reducing plant vigor.
   - *Cecidochares connexa* (Stem-galling fly): Induces galls in stems, restricting nutrient flow and weakening the plant structure.
   - *Caloptilia xanthostigma* (Leaf-mining moth): Its larvae tunnel into leaves, reducing the plant's ability to photosynthesize.
2. **Pathogens**
   - *Puccinia spegazzinii* (Rust fungus): Infects leaves, causing leaf drop and reducing the plant's ability to spread.
   - *Colletotrichum gloeosporioides* (Anthracnose fungus): Causes leaf spots and necrosis, leading to plant dieback.

Effectiveness and Challenges

The introduction of these biological control agents has shown positive results in many countries, significantly reducing *Siam weed* infestations. However, environmental factors such as climate conditions, host specificity, and plant resistance variations can influence their effectiveness.

For long-term management, biological control is often integrated with mechanical and chemical methods. Continued research is essential to identify and enhance the efficiency of biological control agents for sustainable suppression of *Chromolaena odorata* in affected ecosystems.

Biological Control of Crofton Weed (*Ageratina adenophora*)

Crofton weed (*Ageratina adenophora*), also known as *Mexican devil weed*, is an aggressive invasive species native to Central America. It has spread widely in tropical and subtropical regions, forming dense stands that outcompete native vegetation, reduce biodiversity, and affect agricultural productivity. Due to its rapid spread and resistance to mechanical and chemical control methods, biological control has been an effective and sustainable management strategy.

Biological Control Agents

Several biological control agents have been introduced to suppress *Ageratina adenophora* populations:

1. **Insect Herbivores**
   - *Procecidochares utilis* (Gall fly): This is the most widely used biocontrol agent. It induces galls in the stems of *Crofton weed*, restricting nutrient flow and weakening the plant. Over time, this reduces seed production and overall plant vigor.
   - *Lixus aemulus* (Weevil): The larvae bore into stems, damaging vascular tissues and leading to plant dieback.
2. **Pathogens**
   - *Passalora ageratinae* (Leaf-spot fungus): Infects leaves, causing necrotic spots that lead to defoliation and reduced photosynthesis.
   - *Colletotrichum gloeosporioides* (Anthracnose fungus): Causes leaf blight, reducing plant growth and survival.

Effectiveness and Challenges

The introduction of *Procecidochares utilis* has been particularly successful in reducing *Crofton weed* infestations in many countries, including Australia and India. However, the effectiveness of biological control agents can vary due to environmental factors such as climate, plant genetic variations, and interactions with other species.

For sustainable management, biological control is often integrated with mechanical removal and habitat restoration. Ongoing research aims to enhance the effectiveness of biocontrol agents and develop new strategies to suppress *Ageratina adenophora* in affected ecosystems.

Biological Control of *Parthenium hysterophorus*

Parthenium hysterophorus, commonly known as Parthenium weed or Congress grass, is a highly invasive weed that poses severe threats to agriculture, biodiversity, and human health. It spreads rapidly, competes with native vegetation, reduces crop yields, and causes allergic reactions in humans and livestock. Due to its aggressive nature, biological control has been a key strategy for its sustainable management.

Parthenium hysterophorus, commonly known as Congress grass, is a highly invasive weed that has spread extensively across India. Since its first recorded appearance in Maharashtra in 1955, it has rapidly disseminated throughout the country. As of 2009, it was estimated to infest approximately 35 million hectares of land in India. This widespread invasion has led to significant agricultural yield losses, with reductions of up to 40% in several crops, and a 90% decrease in forage production. The weed's proliferation continues to pose a substantial threat to biodiversity, agriculture, and human health across the nation.

Biological Control Agents

Several insect herbivores and pathogens have been introduced to suppress *Parthenium hysterophorus* populations effectively:

1. **Insect Herbivores**
    - *Zygogramma bicolorata* (Parthenium beetle): One of the most effective biocontrol agents, its larvae and adults feed on the leaves, causing defoliation and reducing the plant's ability to reproduce.
    - *Smicronyx lutulentus* (Seed-feeding weevil): Lays eggs in Parthenium flower buds, leading to larval feeding that destroys seeds and reduces the soil seed bank.
    - *Epiblema strenuana* (Stem-galling moth): Larvae bore into the stems, forming galls that stunt growth and reduce flowering.
2. **Pathogens**
    - *Puccinia abrupta var. partheniicola* (Rust fungus): Infects leaves, leading to necrotic lesions that weaken the plant and reduce its spread.
    - *Colletotrichum gleosporioides* (Anthracnose fungus): Causes leaf blight, reducing photosynthesis and plant survival.

Effectiveness and Challenges

Biological control of *Parthenium hysterophorus* has shown promising results in several countries, particularly in Australia and India, where *Zygogramma bicolorata* has significantly reduced Parthenium populations. However, the success of biological control depends on factors such as climate, host specificity, and integration with other control methods.

Biocontrol agents are evolution's own herbicides - targeted, sustainable, and renewable.

PRINCIPLES AND TECHNIQUES FOR MASS MULTIPLICATION OF WEED BIO-AGENTS

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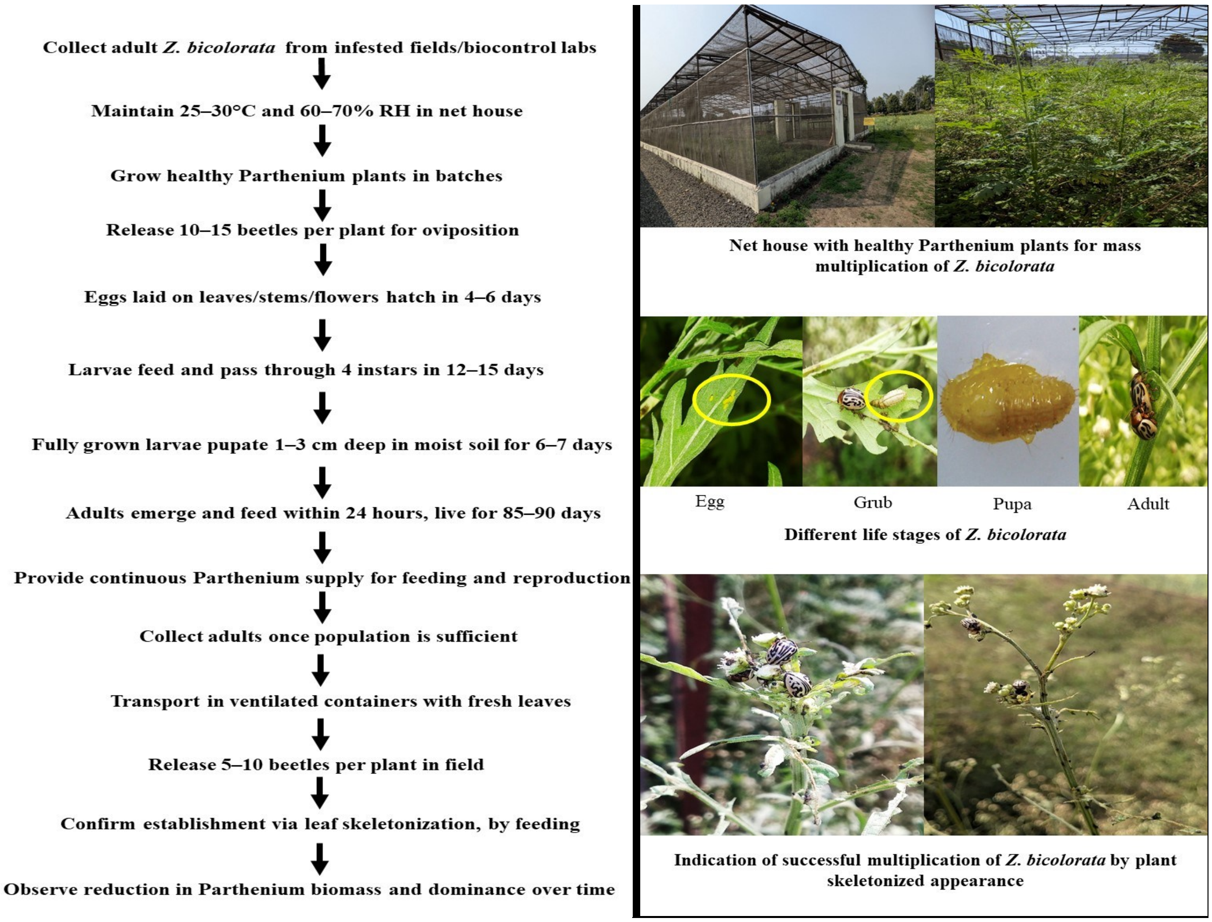
Introduction

The mass multiplication of weed biocontrol agents such as *Zygogramma bicolorata*, *Neochetina eichhorniae*, *Neochetina bruchi*, and *Cyrtobagus salvinae* is essential for the effective management of invasive weeds like *Parthenium hysterophorus*, *Eichhornia crassipes* (water hyacinth), and *Salvinia molesta*. These host-specific agents suppress weed populations by feeding on their tissues, weakening their structure, and reducing their reproductive capacity. The success of mass multiplication depends on maintaining optimal rearing conditions, ensuring a continuous host plant supply, and preventing contamination from predators and pathogens. Proper rearing techniques, large-scale production facilities, and strategic field releases ensure the sustainability and effectiveness of these biocontrol agents in restoring ecological balance and controlling invasive aquatic and terrestrial weeds.

Protocol for net house mass multiplication of *Zygogramma bicolorata*

Parthenium hysterophorus is a highly invasive weed that significantly impacts agricultural productivity and biodiversity. *Zygogramma bicolorata*, a leaf-feeding beetle, has been effectively used as a biocontrol agent for managing *Parthenium hysterophorus*. This protocol outlines the step-by-step procedure for mass multiplication of *Z. bicolorata* in a net house environment. The process begins with the collection of initial stock, where adult beetles are gathered from infested fields or biocontrol laboratories and transported in ventilated containers with fresh *Parthenium* leaves to maintain their health. In a well-ventilated net house with healthy *Parthenium* plants, optimal environmental conditions are maintained at a temperature of 25-30°C and humidity of 60-70%. Fresh *Parthenium* plants are provided in batches to ensure a continuous food supply. Eggs are laid singly or in clusters (4-5 eggs) on leaf surfaces, stems, or flowers. They are small, oblong, smooth, light yellow in color, and have an incubation period of 4-6 days. The larvae pass through four instars over 12-15 days, feeding on *Parthenium* leaves. Newly hatched larvae are pale yellow, turning creamish white, and require a constant fresh leaf supply under hygienic conditions to prevent infections. The pupal stage occurs when fully grown larvae burrow 1-3 cm into the soil, forming spherical earthen cocoons, where they pupate for 6-7 days. Maintaining a moist but not overly wet substrate is crucial for proper pupation. Upon adult emergence, beetles begin feeding on *Parthenium* and can survive for 85-90 days. Once a sufficient population is established, field-ready beetles are prepared for release. Successful multiplication in field is indicated by skeletonization of leaves, where extensive feeding removes leaf mesophyll, leaving only veins, reducing the plant’s ability to photosynthesize. An increased adult population, with high densities of actively feeding, mating, and ovipositing beetles, further confirms successful breeding. Heavy feeding leads to progressive defoliation, wilting, and desiccation of *Parthenium hysterophorus*, ultimately reducing its dominance in infested areas.



Protocol for Mass Multiplication of *Neochetina eichhorniae* and *Neochetina bruchi*

Water hyacinth (*Eichhornia crassipes*) is an invasive aquatic weed that disrupts water ecosystems, but it can be effectively controlled using *Neochetina eichhorniae* and *Neochetina bruchi*, two specialized biocontrol weevils. To mass-rear these weevils, adult specimens are collected from infested water bodies or biocontrol laboratories and transported in aerated containers with fresh water hyacinth leaves. The rearing setup consists of iron tubs (3×6 ft), cement tanks (11×16×8 ft), or artificial ponds with fresh water, maintaining a depth of 2.5 ft for optimal plant growth. Clean, uncontaminated water is essential, and healthy water hyacinth plants serve as the primary food source. Upon introduction, 50-100 adult weevils per square meter are released onto the water hyacinth and housed in insect-proof enclosures to prevent escape. Partial shade is provided, and environmental conditions are maintained at 25-30°C. The biology of *Neochetina* involves eggs being laid inside the petiole, which are whitish, ovoid (~0.75 mm), and hatch within a week at 30°C. The larvae mine toward the petiole base, feeding on the arenchyma, appearing white or cream with a yellow-orange head, and developing through three instars over 6-7 weeks. Pupation occurs underwater, where larvae form root-attached cocoons, from which adults emerge within 3-4 weeks. Adults exhibit sexual dimorphism, with males being smaller (5-6 mm) and brown/tan in color. They feature a V-shaped scale pattern on the elytra and feed on the leaf epidermal tissue without creating holes, while females can lay up to 300 eggs. Successful multiplication of *Neochetina* is indicated by characteristic feeding punctures on leaves, where adult weevils create brown feeding scars that coalesce into necrotic patches. Heavy feeding leads to leaf curling, shriveling, and premature senescence, reducing plant buoyancy and causing mat fragmentation. Additionally, larval mining in petioles and root damage weakens internal transport systems, and pupation in the root zone further compromises plant structure.

Collect adult *Neochetina* sp. from infested water bodies or biocontrol labs

↓

Set up rearing in iron tubs, cement tanks, or artificial ponds (depth ~2.5 ft) with clean water and healthy water hyacinth

↓

Release 50–100 adults/m² into insect-proof enclosures under partial shade at 25–30°C

↓

Adults lay ~0.75 mm whitish, ovoid eggs inside petioles, hatching in ~7 days

↓

Larvae feed internally through 3 instars over 6–7 weeks, mining aerenchyma tissue

↓

Fully grown larvae pupate underwater in root-attached fibrous cocoons

↓

Adults emerge in 3–4 weeks, with males (5–6 mm) smaller and tan-brown with V-shaped elytral markings

↓

Adults feed on leaf epidermis creating brown scars and necrotic patches

↓

Females lay up to 300 eggs

↓

Heavy feeding causes leaf decaying, senescence, reduced buoyancy, and mat fragmentation

↓

Larval tunneling and pupation damage vascular tissues and roots

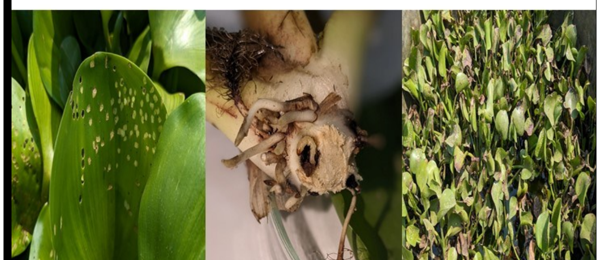
↓

Successful multiplication confirmed by dense adult populations, characteristic feeding scars, and structural weakening of water hyacinth mats.

***Neochetina* sp. rearing Setup: Use iron tubs (3x6ft), cement tanks (11x16x8ft) for growing *E. crassipes* .**

Egg Grub Pupa Adult

Different life stages of *Neochetina* sp.



Indication of successful multiplication of *Neochetina* sp. by circular feeding scars coalescing into larger necrotic patches and larval tunneling into the petioles and rhizomes

Protocol for Mass Multiplication of *Cyrtobagus salvinae*

Salvinia molesta is an invasive floating aquatic plant that disrupts water ecosystems, but its spread can be effectively controlled using *Cyrtobagus salvinae*, a specialized weevil. For mass rearing, adult *C. salvinae* are collected from infested water bodies or biocontrol laboratories and transported in aerated containers with fresh *Salvinia molesta* leaves. The rearing setup consists of iron tubs (3×6 ft), cement tanks (11×16×8 ft), or artificial ponds filled with clean, uncontaminated freshwater to a depth of 2.5 ft. Before introducing weevils, a dense growth of *Salvinia molesta* is established, with environmental conditions maintained at 22-30°C temperature and 70–80% humidity. Partial shade is provided to prevent excessive heat stress. Adult weevils are introduced at a density of 10–20 per square meter, where they feed, mate, and oviposit within submerged plant parts. Overcrowding is avoided to prevent food depletion and competition. The biology of *C. salvinae* follows a structured life cycle: eggs, which are small, white, and oval-shaped, are laid inside *Salvinia* leaf buds and hatch within 5-7 days under optimal conditions. The larvae burrow into plant tissues, feeding internally over three instars in 3-4 weeks, weakening the plant's structure and reducing buoyancy. Mature larvae then move to the roots and rhizomes for pupation, which occurs underwater within plant tissues or root masses, lasting 7-10 days. Adults, which are small (~2 mm), dark brown to black, and nocturnal, emerge and continue feeding on young leaves and buds. They live for 60-100 days, with females laying up to 300 eggs in their lifetime. Successful multiplication of *C. salvinae* is indicated by extensive internal damage, leading to browning and necrosis of plant material. As feeding larvae weaken the rhizomes, petioles, and buds, *Salvinia molesta* loses buoyancy, causing mats to sink beneath the water surface.

Collect adult *C. salvinae* from infested water bodies or biocontrol labs

↓

Prepare rearing setup using iron tubs, cement tanks, or artificial ponds with 2.5 ft depth of clean freshwater

↓

Establish dense *S. molesta* growth under 22–30°C and 70–80% humidity with partial shade

↓

Introduce 10–20 adult weevils/m² to feed, mate, and oviposit within submerged plant parts

↓

Prevent overcrowding to avoid competition and food depletion

↓

Adults lay small white oval eggs in leaf buds, hatching in 5–7 days

↓

Larvae feed internally through three instars over 3–4 weeks, burrowing into plant tissues and reducing buoyancy

↓

Mature larvae move to roots and rhizomes for pupation, occurring underwater within plant tissue over 7–10 days

↓

Small (~2 mm), dark brown to black nocturnal adults emerge and feed on young buds and leaves

↓

Adults live for 60–100 days, with females laying up to 300 eggs

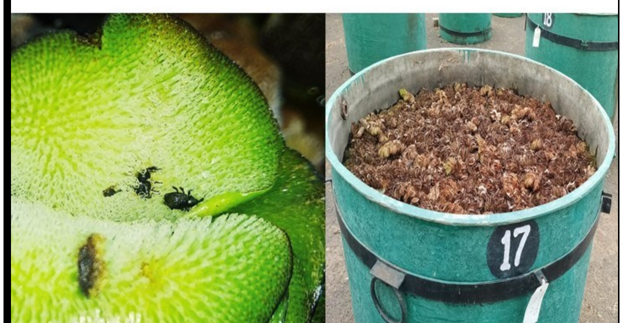
↓

Successful multiplication marked by internal plant damage, leaf browning, rhizome weakening, and sinking of *Salvinia* mats due to loss of buoyancy.

C. salvinae rearing Setup: Use iron tubs (3x6ft), cement tanks (11x16x8ft) for growing *S. molesta*.

Egg Grub Pupa Adult

Different life stages of *C. salvinae*



Indication of successful multiplication of *C. salvinae* by browning and necrosis of *S. molesta*

Critical Considerations for Effective Biocontrol Agent Multiplication

- Successful multiplication of biocontrol agents requires careful selection of a host-specific (monophagous) species to ensure targeted pest suppression without harming non-target organisms.
- Maintaining an optimal rearing environment, including proper temperature, humidity, and light conditions, is crucial for healthy population growth.
- A continuous and sufficient supply of host plants must be ensured to support all life stages of the biocontrol agent, while maintaining genetic diversity and preventing inbreeding to sustain a robust population.
- Regular monitoring is essential to track population growth and detect potential contamination from predators or pathogens that could compromise the rearing system.
- For sustainability and large-scale production, training field staff and farmers in mass rearing techniques and implementation strategies is vital for widespread adoption.
- An effective field release strategy involves introducing bio-agents at their optimal stage and at recommended densities to maximize their establishment and impact in controlling invasive species.

Mass rearing is not just production- it is the art of nurturing nature for a purpose.

HARNESSING NATURE'S ARSENAL: DEVELOPING AND DEPLOYING MICROBIAL HERBICIDES FOR SUSTAINABLE WEED CONTROL

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Introduction

Weeds are a major agricultural pest, affecting crop yields by over 34% globally. They compete with desired plants and threaten food supplies and ecosystems. Controlling weeds is crucial for agricultural output and a major agricultural ecosystem issue. Chemical weed management, such as herbicides, costs over $14 billion a year. Chemical weed management is not the best choice for organic cropping techniques due to environmental concerns. Researchers are developing sustainable, protective weed control methods, such as biological weed control, which uses natural enemies like insects and pathogens. Inundative bioherbicide techniques are one such method.

Basis of Bioherbicide approach

Biological control is the deliberate use of natural enemies to reduce the population of a target weed to below a desired threshold and can be divided into two main approaches:

Classical (inoculative) Approach: Inoculative biocontrol is a traditional method that uses imported diseases to manage native or naturalized weeds. It targets exotic weeds without natural enemies, releasing aggressive host-specific viruses that restrict the weed's population.

Bioherbicide (inundative) approach: Bioherbicides are an inundative method that uses native plant diseases isolated from weeds to eradicate specific weeds before they cause financial harm. They can be used in both cultivated and natural environments, and can be applied as granules or sprays using standard pest control tools.

i Definition

Bioherbicides are herbicides based on natural biological agents like fungi, bacteria, viruses, protozoans, and nematodes. Microbial bioherbicides are phytopathogenic microorganisms used for biological weed control, with 60% derived from fungal and bacterial strains. Bioherbicides are formulated to avoid unfavorable environmental conditions and facilitate application. The development of an effective bioherbicide requires a thorough understanding of the pathogen(s), target weed biology and population dynamics, optimal disease initiation and development requirements, and complex interactions within the host-pathogen system.

1. History of Microbial Bioherbicides

Biological weed control methods have been used for over 200 years, using herbivorous animals and pathogenic microbes. The concept of bioherbicides gained significant interest after World War II, with early experiments using fungi like *Fusarium oxysporum* and *Alteraria cuscutacidae*. Official development began in the late 1960s, focusing on pathogens for various plants. Chinese researchers produced "LuBao" in 1963, which remains in use today. The mid-1970s saw mycoherbicide development, leading to the global registration of numerous bioherbicides, such as De Vine and Collego.

Table 1: List of registered bioherbicides and their respective sources, target weeds, ecosystems

Microorganisms	Target Weeds	Registered Name	Status	Ecosystems
Cephalospprium diospyri	*Diospyras virginiana* L.	Oklahoma	-	Pastures, rangelands
Colletotrichum gloeosporioides f. sp. aeschynomene	*Aeschynomene virginica* L.	Collego™	Commercialized	Rice, soybean
Alternaria cassiae	*Cassia obtusifolia* L.	'CASST'	Formulation development	Soybean
Phytophthora palmivora	*Morrenia odorata* (Hook. &Arn.) Lindl.	Devine™	Commercialized	Citrus groves
Xanthomonas campestris	*Poa annua* L.	Camperico®	Commercialized	Turf, athletic fields
Cylindrobasidiumleave	*Acacia* spp	Stump-Out™	Commercialized	Forest, rangelands
Colletotrichum gloeosporioides	*Hakea sericea* Schrad. & J. C. Wendl.	Hakak	Commercialized	Mountain meadows
Colletotrichum gloeosporioidesmalvae	*Malva pusilla* Sm.	BioMal®	Commercialized	Flex, lentil, horticultural crops
C. purpureum	*P. serotina*	Biochon™	Commercialized	Forest
Phoma macrostoma	*Reynoutria japonica* Houtt.	Phoma	Commercialized	Golf courses, agriculture, and agro-forestry
Streptomyces acidiscabies	*Taraxacum officinale* L.	Opportune®	Commercialized	Turf
Alternaria destruens	*Cuscuta* spp.	Smolder	Field evaluation	Cranberry
Chondrostereum purpureum (Fr.) Pouz	*Prunus serotina* Ehrh.	Mycotech™	Commercialized	Forest, mountains
C. purpureum	*Populus euramericana* Guinier	Chontrol®	Commercialized	Forest
Sclerotinia minor Jagger.	*Taraxacum* spp.	Sarritor®	Commercialized	Turf
Puccinia thlaspeos C. Shub.	*Isatis tinctoria* L.	Woad Warrior®	Commercialized	Forest, rangelands, pastures

2. Mechanisms of Microbial Action

Bioherbicides have emerged as a promising alternative for effectively controlling weeds through various mechanisms. Several common modes of action have been identified.

a. ***Growth inhibition:*** Bioherbicides disrupt weed growth by targeting essential proteins or hormones within the thylakoid membrane, causing significant slowdown or cessation of weed development.

b. ***Cell structure disruption:*** Bioherbicides damage weed cell structure, increasing susceptibility to microorganisms and desiccation, causing weakened weeds to struggle to survive and reproduce effectively.

c. ***Metabolic process interference:*** Bioherbicides disrupt weed metabolism by targeting mitochondria, affecting nutrient conversion and vital functions, hindering growth and spread by debilitating the weed.

d. ***Disease induction:*** Bioherbicides use living microorganisms to induce diseases in weeds, causing them to weaken or kill. The mode of action depends on the substance or microorganisms, targeting specific weed types while minimizing environmental impact. These methods disrupt weed growth, damage cells, and reduce weed survival.

3. Preferred characteristics of a potential bioherbicide microbe

Growth and sporulation on artificial media, highly virulent, genetic stability, restricted host range, broad tolerance range, prolific propagule production, capacity to damage its host plant, and, innocuous in ecological effects.

4. Steps in bioherbicide development

a. Microbial bioherbicides based on cell-free metabolites

Phase I: This involves the isolation and screening of microorganisms capable of combating specific weed species, either from damaged weed areas or soil rhizosphere levels.

Phase II: The strain or strains with the best results after screening will be selected and subjected to a metabolite characterization process, including elucidation of mode of action.

Phase III: The bioherbicide product's efficacy and spectrum of weed species will be evaluated in greenhouses, with toxicity assays focusing on crops related to the target weed. The herbicide production process will be optimized, and a comprehensive formulation will be developed.

Phase IV: The bioherbicide will be produced on a large scale through fermentation, and the metabolites will subsequently be purified for field testing to corroborate their efficacy. Only then can the product be registered, patented, and finally commercialized.

b. Flow Chart for scale-up fermentation for development of microbial bioherbicides based on cell-free metabolites from fungi

PREPARATION OF SEED CULTURE

Prepare PDA plates

↓

Spread 100 **μ**L of fungal spore stock solution over the plate (incubate at 25–28 °C for 7 days**)**

↓

Cut the agar plugs into small pieces (0.5 squares each piece) under aseptic conditions

↓

transfer 15–20 pieces to a sterile 500 mL flask containing 200 mL of PDB, and incubate at 25–28 °C on a rotary

↓

shaker (150–220 rpm) for 5–7 days) to obtain the seed culture

↓

LIQUID-STATE FERMENTATION

Prepare a sterile 500 mL flask containing 250 mL of PDB

↓

Transfer 10 mL of the fungal seed culture into the flask and incubate at 25–28 °C on a rotary shaker (150–220 rpm) for 7–10 days

↓

EXTRACTION OF SECONDARY METABOLITES

Filter the crude extract from biomass using sterile cheese cloth layers or vacuum membrane filtration and transfer to a separating funnel

↓

Add equal volume of ethyl acetate, homogenize the mixture

↓

Let the mixture stand until the organic phase and aqueous phase completely separate; this usually takes about 6–8 h (overnight) to 24 h

↓

Recover the organic phase (upper phase). Add 100 mL of ethyl acetate to re-extract for 4–5 times and pool all extracts together

↓

Evaporate the organic phase under reduced pressure (using a rotary evaporator) at a temperature below 40 °C till 8–10 mL organic phase is left

↓

Transfer it into another weighed microfuge tubes and evaporate with a gentle stream of nitrogen gas to obtain the dry metabolites

↓

Re-dissolve the dry metabolites with 8–10 mL ethyl acetate/methanol and transfer it to microfuge tubes to obtain a crude extract

↓

The extract is fractionated by thin layered chromatography (TLC) using petroleum ether–EtOAc gradient elution or specific solvents

↓

The fraction eluted with 30% EtOAc is separated by column chromatography (CC) (200–300 mesh) using 1:1 CH_2Cl_2–MeOH as eluent

↓

Each of the resulting sub-fractions is combined and further separated by a preparative column (RP-C18 column, Agilent ZORBAX, 9.4 × 250 mm, 5 µm; 50% MeCN in H_2O for 2 min, followed by 50–55% MeCN for 25 min; 2 mL/min) to obtain the pure metabolite

↓

Store the pure metabolite for further analyses by liquid chromatography–mass spectrometry (LC/MS) and nuclear magnetic resonance (NMR) to identify the accurate structure of the metabolite

c. *Methods for evaluation of herbicidal or phytotoxic properties of microbes or their metabolites*

Bioassays*:* Seed germination, seedling, and detached leaf assay

Biochemical screening**:** Estimation of hydrolytic enzymes, defence related enzymes, phenols, flavonoids, amino acids, antioxidants, phytohormones

5. Techniques and approaches for bioherbicide application

The effectiveness of bioherbicides depends on their application method, influencing both weeds and crops. Proper usage, following guidelines, and selecting the right application technique are crucial for successful weed management. Bioherbicides play a significant role in integrated weed control strategies.

- **Foliar Application** – This common method involves spraying bioherbicides directly onto weed leaves, ensuring absorption through the foliage and stem. The bioherbicide then spreads to the roots, hindering weed growth.

- **Soil Application** – Bioherbicides are applied to the soil around crops, either in liquid, granular, or powdered form. Mixing them into the soil through tilling or watering enhances their contact with weed seeds and seedlings, preventing their development.
- **Seed Treatment** – Seeds are coated with bioherbicides before planting, offering early protection against weed competition by limiting weed growth near germinating crops.
- **Pre-emergence & Post-emergence Application** – Pre-emergence treatment applies bioherbicides before weeds sprout, while post-emergence treatment targets visible weeds directly.
- **Spot Treatment** – A selective method where bioherbicides are applied only to specific weed-infested areas, reducing unnecessary exposure to crops and improving efficiency.

Each method has its advantages, and choosing the right approach enhances the effectiveness of bioherbicides in sustainable weed control.

6. Constraints and limitations of bioherbicide applications

Except the numerous advantages of bioherbicides, some circumstances have been noted to restrain the progress of bioherbicides into profitable outputs. These include:

a. ***Biological restrictions***: Host changeability, host scope resistance mechanisms and interaction with other microorganisms that affect efficacy.
b. ***Environment restrictions***: Epidemiology of bioherbicide reliant on optimal environmental conditions
c. ***Technical restrictions***: Wholesale production and formulation development of reliable and effective bioherbicide.
d. ***Commercial restrictions***: Market capacity, patent protection, confidence and adjustment.

Microbes are nature's chemists- why not let them formulate our herbicides.

UTILIZATION OF INVASIVE WEEDS

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Introduction

Invasive weeds are plant species that spread aggressively, outcompeting native flora and causing ecological, economic, and health problems. Their proliferation can lead to reduced soil fertility, biodiversity loss, and increased management costs. However, rather than viewing them solely as a problem, these weeds can be utilized in various ways to provide economic and environmental benefits. This lecture explores the innovative utilization of invasive weeds, highlighting their applications in biofuels, agriculture, industry, and environmental remediation.

Understanding Invasive Weeds

Invasive weeds are non-native plants that establish themselves in new environments, often displacing native species. They can spread rapidly due to their high adaptability, lack of natural predators, and ability to thrive in diverse conditions. Some common characteristics include:

- High reproductive capacity, allowing them to multiply rapidly.
- Resistance to pests and diseases, ensuring their survival.
- Efficient seed dispersal mechanisms, which help them spread quickly.

The Problem of Invasive Weeds

1. Environmental Impact

- **Displacement of Native Species**: Invasive weeds reduce biodiversity by competing with native flora for nutrients, sunlight, and space.
- **Soil Degradation**: Many invasive species alter soil composition, reducing its fertility and making it unsuitable for native plants.
- **Impact on Water Bodies**: Aquatic invasive weeds such as *Eichhornia crassipes* (water hyacinth) create dense mats on water surfaces, reducing oxygen levels, blocking sunlight, and harming aquatic life.
- **Forest and Grassland Disruption**: Certain invasive species form dense thickets, preventing the natural regeneration of forests and reducing grazing areas for wildlife and livestock.

2. Health Impact

- **Allergies and Respiratory Issues**: Some weeds, like *Parthenium hysterophorus*, release airborne pollen that causes severe allergies and respiratory problems.
- **Toxicity to Livestock**: Some invasive weeds contain toxic compounds that can be harmful to animals when ingested in large quantities.
- **Human Health Hazards**: Prolonged exposure to invasive plant sap or pollen can cause skin irritation, dermatitis, and other health issues.

3. Economic Impact

- **Increased Management Costs**: Farmers and landowners spend significant resources on controlling invasive weeds through herbicides, mechanical removal, and biological control measures.
- **Reduced Agricultural Productivity**: Invasive weeds compete with crops for nutrients, water, and sunlight, leading to lower yields and economic losses.
- **Impact on Infrastructure**: Overgrowth of invasive weeds in irrigation channels, roadsides, and urban areas leads to additional maintenance costs.

Why Utilize Invasive Weeds?

Instead of viewing invasive weeds as a threat, they can be transformed into valuable resources. Benefits of utilizing invasive weeds include:

- **Sustainability**: Utilizing these weeds for productive purposes reduces their negative impact on ecosystems.
- **Economic Benefits**: Industries can be developed around the commercial use of invasive weeds, creating employment and business opportunities.
- **Waste Management**: Instead of disposing of these plants as waste, they can be converted into biofuels, compost, and other useful products.

Environmental Benefits of Utilizing Invasive Weeds

- **Reduction in Weed Spread**: Harvesting these plants for commercial use helps control their spread.
- **Soil Fertility Improvement**: Some invasive weeds, when processed properly, can be used to enhance soil quality by adding organic matter and nutrients.
- **Carbon Sequestration**: Fast-growing weeds can absorb significant amounts of carbon dioxide, contributing to climate change mitigation.

Specific Uses of Invasive Weeds

1. *Parthenium hysterophorus*

- **Biofuel & Biomass Production**: The plant can be converted into bioethanol and biogas, providing an alternative energy source.
- **Medicinal Applications**: Contains bioactive compounds with anti-inflammatory, antibacterial, and antifungal properties.
- **Composting**: Can be used to create organic compost, improving soil health.
- **Handmade Paper Production**: The fibrous nature of *Parthenium* makes it suitable for biodegradable paper production.

2. *Lantana camara*

- **Biofuel & Biomass Production**: Used to make briquettes and biogas for energy generation.
- **Handicrafts**: The strong, flexible stems are ideal for making furniture, baskets, and ropes.
- **Essential Oils**: Extracts have insecticidal and medicinal properties.
- **Livestock Bedding**: Processed *Lantana* stems can be used as animal bedding.

3. *Eichhornia crassipes* (Water Hyacinth)

- **Water Purification**: Absorbs heavy metals, phosphates, and nitrates from polluted water.

- **Agricultural Uses**: Used as organic mulch and compost to enhance soil fertility.
- **Industrial Uses**: Paper and textile industries utilize its fibers for eco-friendly production.

4. *Salvinia molesta*

- **Phytoremediation**: Absorbs excess nutrients and pollutants from contaminated water.
- **Oil Spill Cleanup**: Effective in absorbing oil spills, aiding in environmental cleanups.
- **Livestock Feed**: After proper treatment, it can be mixed into animal feed.

Case Studies of Aquatic Weed-Based Phytoremediation

- **Urdua Village, Jabalpur**: A phytoremediation project using *Eichhornia crassipes* and *Pistia stratiotes* successfully treated contaminated water, showing significant reductions in turbidity, sodium, chloride, sulfate, and phenol levels.
- **Arsenic Removal in Water**: A study found that a combination of *Typha latifolia*, *Eichhornia crassipes*, and *Hydrilla verticillata* reduced arsenic levels by over 99% in just 15 days.
- **Impact on Agriculture**: Phytoremediated water used for irrigation in spinach cultivation resulted in safe arsenic levels in harvested crops, ensuring food safety.

Challenges in Utilizing Invasive Weeds

- **Public Perception**: Many invasive plants, such as *Parthenium*, have a negative reputation due to their toxicity.
- **Economic Constraints**: Processing these weeds into usable products requires investment in technology and infrastructure.
- **Risk of Further Spread**: If not managed properly, utilizing invasive weeds may inadvertently contribute to their propagation.

Conclusion

The utilization of invasive weeds presents an opportunity to turn an ecological challenge into an economic and environmental benefit. From biofuels and phytoremediation to handicrafts and agriculture, these plants offer multiple applications. However, careful management is necessary to prevent their further spread and maximize their potential. By integrating invasive weed utilization into ecological restoration and industrial processes, we can create a sustainable approach to biodiversity conservation and resource efficiency.

The future belongs to those who turn ecological threats into bioeconomic solutions

RESOURCE PERSON

Dr. M. Sampath Kumar is currently working as Senior Scientist (Agricultural Entomology), Division of Germplasm Collection and Characterization, ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. He is also involved in the quarantine activities of the Bureau. He dedicatedly involved in importation of classical biocontrol agents for the management of invasive insect pests and weeds.

Dr. Puja Ray is an Assistant Professor at Presidency University, Kolkata, India. She has a diverse research interest includes environmental impact assessment of invasive plant species and their interactions with their insect and fungal biocontrol agents and native biodiversity. Most of her earlier research background has revolved integrated weed management

Dr. Sushil Kumar joined the Agricultural Research Service in 1991 and ICAR-DWR in 1994. He has made significant contributions to biologically based integrated management and utilization of terrestrial and aquatic weeds, particularly Parthenium, water hyacinth, and *Salvinia molesta*. He has published over 100 papers in reputed journals, along with several bulletins and books, including *Fifty Years of Weed Research in India*.

Dr. Archana Anokhe working as a scientist, ICAR-Directorate of Weed Research, Jabalpur. Previously she worked in ICAR- DRMR, Bharatpur & IARI, New Delhi and worked in area such as host-plant interaction in oilseeds as well as on enhancing the efficacy of BT and its utilization as a endosymbionts. Currently she is working on biocontrol management of invasive weeds & development of quarantine facilities at ICAR- DWR.

Dr. Deeksha M. G. is scientist at ICAR-DWR, Jabalpur. She was a visiting scholar at University of Queensland, Australia in 2023, and has received DST-inspire fellowship. Specialized in Insect pollination ecology, insect molecular biology and biochemistry. Currently, she works on biocontrol management of invasive weeds at ICAR-DWR.

Dr. Himanshu Mahawar is working as a scientist at ICAR-Directorate of Weed Research, Jabalpur. His research interest includes assessment of environmental impact of herbicides in rice-wheat cropping system under conservation agriculture on soil microbial community and structure. His current research projects have been on biological management of major weeds in rice-wheat cropping system plant pathogens/microorganisms or their products.

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ISBN : 978-81-985626-3-0