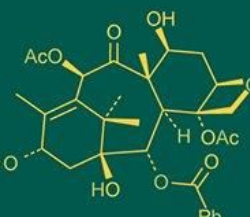
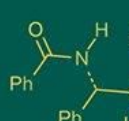


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Evaluation of mycoherbicide formulation for the management of water hyacinth [*Eichhornia crassipes* (Mart.) Solms]

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Abstract

The fungus *Alternaria eichhorniae* is being developed as a mycoherbicide to control water hyacinth [*Eichhornia crassipes* (Mart.) Solms]. Inoculum concentrations above 2% were all equally effective in achieving 100% weed control. Initially, spores or fungal propagules (0 days old) were more virulent than older ones (4 months old). Talc-based formulations of *A. eichhorniae* were evaluated under natural outdoor conditions in pots. These formulations, developed from pure cultures, allowed spores to germinate and infect the weed under environmental conditions. All formulations induced higher levels of disease in water hyacinth plants. Reducing the talc powder in the dust formulation from 50% to 10% did not affect efficacy. The most virulent fungi *A. eichhorniae* showed a damage intensity of 85.06% with 2.1×10^7 cfu per gram at 15 days after spraying at a 2% concentration. These results confirm the utility and feasibility of talc-based mycoherbicide formulations that promote the bioherbicide's activity.

Keywords: Mycoherbicide, *A. eichhorniae*, biological control, PDA, concentration

Introduction

Water hyacinth [*Eichhornia crassipes* (Mart.) Solms], an invasive aquatic plant native to the Amazon basin, was introduced to India in 1896 as an ornamental species. It has since proliferated across various water bodies, including those in Rajasthan, notably in Udaipur's Pichhola Lake, Ayad River, and Swaroop Sagar. By 2022, infestations had expanded to over 455,000 hectares, affecting more than 350 districts nationwide. This weed forms dense mats that obstruct sunlight and deplete oxygen levels, disrupting aquatic ecosystems. Its rapid growth hampers irrigation, hydroelectric power generation, and navigation, while also promoting mosquito breeding and accelerating water evaporation. Traditional control methods like manual removal are labour-intensive and costly, and chemical herbicides pose environmental and health risks. Biological control has emerged as a promising alternative. Among the fungi *Cercospora rodmanii* Conway has shown the greatest promise as a biocontrol agent of water hyacinth (Conway, 1976) [3]. Studies conducted by Santhi (1994) [12] and Susha (1997) [14] revealed that *Fusarium pallidorozeum* (Cooke) Sacc., *Fusarium equiseti* (Corda) Sacc., *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and *Alternaria eichhorniae* (Shabana, 1995) have demonstrated efficacy in suppressing water hyacinth growth. These fungi can be formulated into mycoherbicides that are environmentally friendly and cost-effective. Integrating these biological agents with physical and chemical methods offers a sustainable approach to managing water hyacinth infestations and mitigating their impact on water resources and ecosystems.

Materials and Methods

Preparation of talc-based mycoherbicide formulation

A talc-based formulation products of the fungi (*Cercospora rodmanii*, *Alternaria eichhorniae*, *Fusarium pallidorozeum*) was prepared, as all these have maximum intensity of infection.

For the preparation of inoculum, pure culture of *C. rodmanii*, *A. eichhorniae*, *F. pallidorozeum* were maintained on PDA discs of 5mm diameter from seven-day old culture

of *C. rodmanii*, *A. eichhorniae*, *F. pallidoroseum* were inoculated into 100 ml sterilized potato dextrose agar broth in 250ml conical flasks. The flasks were incubated at room temperature (28 ± 2 °C) for 10 days.

At the end of the incubation period, the mycelium was separated from the broth by filtering through filter paper. The fungal mat thus obtained was pressed between layers of sterile coarse filter paper to remove water and thoroughly ground using a blender.

A wettable powder formulation of the mycelium was prepared with the following composition: fungi mycelium-40 g, talc-54 g, sucrose-2 g, tween 80-2 g and glycerol-2 g. After proper blending, the wettable powder formulation was air dried and stored in polythene bags at room temperature (28 ± 4 °C) and under refrigeration (4 °C) (Naseema *et al.*, 2004) [9].

Evaluation of mycoherbicide formulation for management of water hyacinth

Different talc-based mycoherbicide formulations and chemical weedicide were evaluated for the management of water hyacinth.

Treatment details:

Treatment:-5 (Five)

T₁ *Cercospora rodmanii* mycoherbicide formulation @ 2 percent,

T₂ *Alternaria eichhorniae* mycoherbicide formulation @ 2 percent,

T₃ *Fusarium pallidoroseum* mycoherbicide formulation @ 2 percent,

T₄ Chemical weedicide (glyphosate 41% SL) @ 0.10 percent and

T₅ control

Replication:-4

Design:-CRD

Water hyacinth plants were transplanted in plastic pots filled with autoclaved Garden-soil-FYM mixture (3:1), with five plant per pot were maintained with three replications.

The different talc-based mycoherbicide formulations were spray inoculated on transplanted water hyacinth plants using a hand automizer sprayer @ 2 percent concentration for mycoherbicide formulation and 0.10 percent for chemical weedicide glyphosate. Water hyacinth plants sprayed with sterile water served as control. The plants were covered with polythene sheet to maintain sufficient humidity. Intensity of infection was recorded using disease rating scale (0-9) at 7, 11 and 15 DAS (Days after spray) and PDI was calculated using the formula given by (Wheeler 1969) [17].

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of all individual disease numerical rating}}{\text{Total number of plants assessed} \times \text{Maximum disease rating scale}} \times 100$$

Table 1: Disease rating scale

Grade/Score	Description
0	No symptoms
1	1-10 percent symptoms development around the pinpricked area only
2	11-20 percent leaf area showing yellowing/browning symptoms
3	21-30 leaf area showing yellowing/browning symptoms
4	31-40 percent of leaf area including petiole showing symptoms
5	41-50 percent of leaf area including petiole showing symptoms
6	51-60 percent of leaf area including petiole showing symptoms
7	61-75 percent of leaf area including petiole showing symptoms
8	More than 75 percent of leaf area
9	Complete drying of plant

To study the shelf life of bio-formulations

In order to find out the shelf life of talc-based bio formulation, the product was filled in sterilized polythene bags @ 500 g, sealed and stored at different temperatures 10 °C, 25 ± 2 °C and 35 °C. Viability of the fungal propagules (spore, mycelium, etc.) were examined at monthly intervals by enumerating the number of colony forming units (cfu) in one-gram of talc-based mycoherbicide formulation by serial dilution technique.

Experimental result

Development of talc-based mycoherbicide formulations

Mycoherbicide formulations of three most virulent fungi were prepared by following the method and composition given by Naseema *et al.*, (2004) [9] i.e., fungi mycelium-40 g, talc-54 g, sucrose-2 g, tween 80-2 g and glycerol-2 g. After proper blending, standard cfu/g of *Cercospora rodmanii* (1×10^6 - 10^8), *Alternaria eichhorniae* (2.1×10^7) and *Fusarium pallidoroseum* (3.0×10^6) were maintained in formulation, packed in polythene bags and stored in

refrigerator (10 °C) (Plate 1).

Evaluation of talc-based mycoherbicide formulations

The talc-based mycoherbicide formulations were evaluated by spraying 2 percent with sticker (*Chipko*) @ 1.0 ml per liter on water hyacinth. The disease intensity (PDI) was recorded at 7 DAS, 11 DAS and 15 DAS (Table 1, Plate 1). Among the formulations, maximum disease intensity (76.03%) at 7 DAS, 79.40 percent at 11 DAS and 85.06 percent at 15 DAS was caused by *Alternaria eichhorniae*, followed by *Cercospora rodmanii* with PDI 64.90 percent at 7 DAS, 69.80 percent at 11 DAS and 75.20 percent at 15 DAS and minimum disease intensity (53.10%) at 7 DAS, 56.20 percent at 11 DAS and 62.20 percent at 15 DAS by *Fusarium pallidoroseum* (Table 1, Plate 1).

Chemical weedicide (glyphosate) @ 0.10 percent caused 100 percent damage at 7 DAS (Table 1, Plate 1). However, *Alternaria eichhorniae* talc-based mycoherbicide formulation was found significantly effective compared to other bio-formulations and next to chemical weedicide.

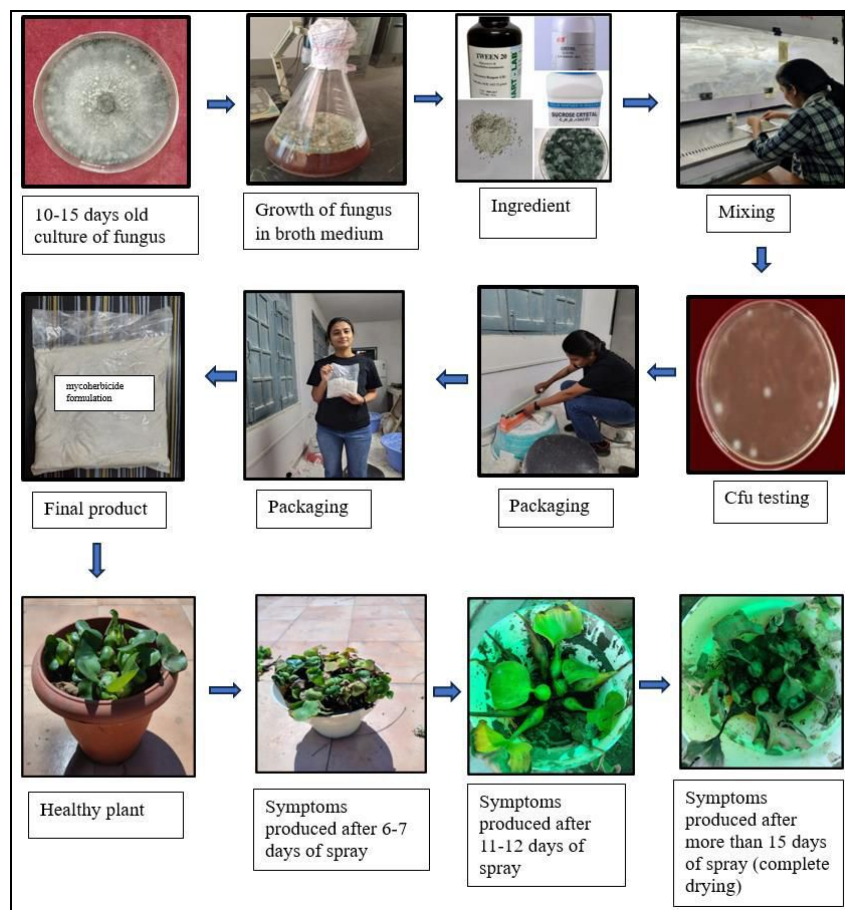


Plate 1: Preparation and evaluation of mycoherbicide formulation on management of water hyacinth

Table 1: Effect of different mycoherbicide formulations on water hyacinth

S. No.	Formulations	Dose	*Disease intensity on water hyacinth (%)		
			7 DAS	11 DAS	15 DAS
1	<i>Cercospora rodmanii</i>	2.0%	64.90 (53.64)	69.80 (56.64)	75.20 (60.10)
2	<i>Alternaria eichhorniae</i>	2.0%	76.03 (60.66)	79.40 (62.98)	85.06 (67.24)
3	<i>Fusarium pallidorozeum</i>	2.0%	53.10 (46.75)	56.20 (48.54)	62.20 (52.04)
4	Chemical weedicide (glyphosate)	0.10%	100 (92.00)	100 (92.00)	100 (92.00)
5	Control (water spray)	-	0	0	0
SEM±			0.87	1.17	1.01
C.D. at 5%			2.80	3.76	3.23
C.V.%			2.78	3.55	2.80

Note: Average of three replications; Figures in parentheses are arcsine $\sqrt{\text{percent angular transformed values}}$. DAS = Days after spray

Studies on shelf life of mycoherbicide formulations

The talc-based mycoherbicide formulations of *A. eichhorniae*, *C. rodmanii* and *F. pallidorozeum* were studied for shelf life at different temperatures. The formulations were filled in sterilized polythene bag and stored at different temperatures 10 °C, 25±2 °C and 35 °C for four months. Viability of the fungal propagules were tested at monthly interval by estimating the number of colony forming units in one-gram (cfu/g).

The viability of the fungal propagules decreased as the storage time increased. Initially, standard cfu of *A. eichhorniae* bio-formulations at all storage temperature was

2.14×10⁶ per gram. At 10 °C viability of spores/fungal propagules were 2.12×10⁶ cfu/g in 1-month of storage, 2.10×10⁶ cfu/g in 2-month of storage, 2.08×10⁶ cfu/g in 3-month of storage and 1.98×10⁶ cfu/g in 4-month of storage. So, that product stored at 10 °C have no extreme decline in cfu/g (Table 2, Plate 2).

However, at 25±2 °C viability of *A. eichhorniae* propagules were decreased from 2.14×10⁶ cfu/g to 0×10⁶ cfu/g in 4-month of storage, 2.09×10⁶ cfu/g in 1-month, 1.52×10⁶ cfu/g in 2-month and 0×10⁶ cfu/g in 3-month. Although at 35 °C gradual reduction in the viability of propagules was reduced to 1.05×10⁶ cfu/g in 1-month, thereafter, a sudden decline in the viability was recorded to 0×10⁶ cfu/g in 3-month and 4-month of storage (Table 2, Plate 2).

The viability of *Cercospora rodmanii* propagules were decreased from 1.6×10⁶ cfu/g to 1.50×10⁶ cfu/g at 10 °C at four months of storage. While at 25±2 °C viability of spores/ fungal propagules initially 1.6×10⁶ cfu/g but gradually decreased from 1-month of storage 1.35×10⁶ cfu/g to 1.02×10⁶ cfu/g in 2-month and in 3 & 4 month it was recorded 0×10⁶ cfu/g. At the temperature 35 °C, initially cfu/g was 1.6×10⁶ and declined to 0×10⁶ cfu/g at 4 months of storage (Plate 2).

In *Fusarium pallidorozeum* mycoherbicide formulation, viability of fungal propagules/spores were minimum decreased when stored at 10 °C. However, at room temperature 25±2 °C, the viability of propagules was 3.21×10⁶ cfu/g at 0 day, 2.96×10⁶ cfu/g in 1-month, 1.56×10⁶ cfu/g in 2-month and in 3 & 4 month it was 0×10⁶ cfu/g. Although at 35 °C, the cfu 1.02×10⁶ cfu/g was at 1-month of storage and declined to 0×10⁶ cfu/g in 2 months of storage (Plate 2).

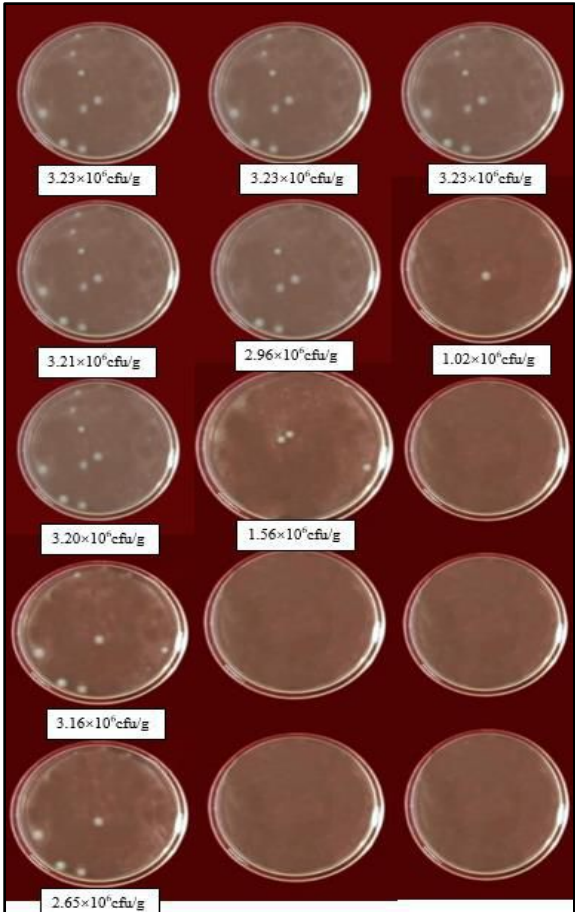


Plate 2: Shelf life of *Fusarium pallidoroseum* mycoherbicide formulation stored at different temperature

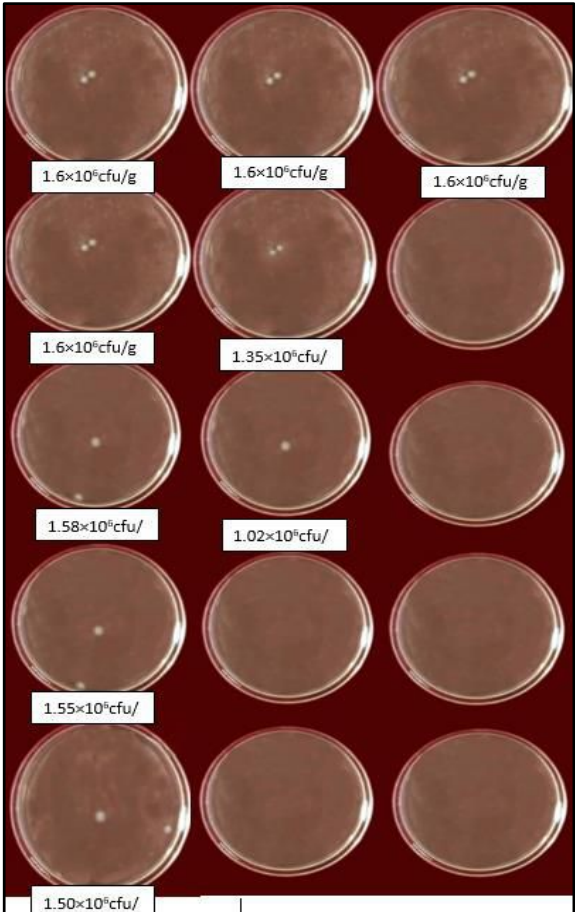


Plate 2: Shelf life of *Fusarium pallidoroseum* mycoherbicide formulation stored at different temperature

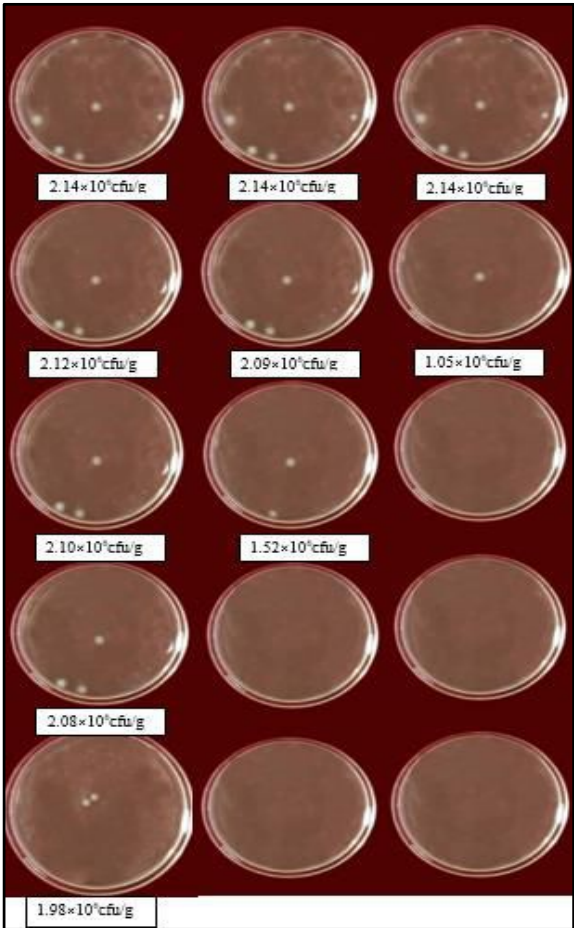


Plate 2: Shelf life of *Cercospora rodmanii* mycoherbicide formulation stored at different temperature

Table 2: Shelf life of mycoherbicide formulations stored at different temperature

S. No.	Storage months	<i>Alternaria eichhorniae</i>			<i>Cercospora rodmanii</i>			<i>Fusarium pallidoroseum</i>		
		*Mean cfu/g × 10 ⁶			*Mean cfu/g × 10 ⁶			*Mean cfu/g × 10 ⁶		
		10 °C	25±2 °C	35 °C	10 °C	25±2 °C	35 °C	10 °C	25±2 °C	35 °C
1	0	2.14	2.14	2.14	1.6	1.6	1.6	3.23	3.23	3.23
2	1	2.12	2.09	1.05	1.6	1.35	0	3.21	2.96	1.02
3	2	2.10	1.52	0	1.58	1.02	0	3.20	1.56	0
4	3	2.08	0	0	1.55	0	0	3.16	0	0
5	4	1.98	0	0	1.50	0	0	2.56	0	0

*Average of three replications

Discussion

Many foliar pathogens need several hours of surface moisture on plants to enable spore germination, the development of infection structures, and successful infection. Developing suitable formulations and application methods for potential mycoherbicides can help address certain environmental and biological challenges. Typically, mycoherbicide formulations involve mixing fungal spores with inert carriers, such as diluents or surfactants, to modify their physical properties for improved usability and handling.

A talc-based combination product of the selected pathogens viz., *Alternaria eichhorniae*, *Cercospora rodmanii*, and *Fusarium pallidoroseum* was prepared by mixing their mycelial mats with fungi mycelium-40 g, talc-54 g, sucrose-2 g, tween 80-2 g and glycerol-2 g. The research studies were conducted to select the most virulent mycoherbicide formulation of fungal pathogens on water hyacinth, the intensity of infection was recorded at 7 days, 11 days and 15 days after spraying. The formulated product varied in their intensity of infection to water hyacinth. The maximum intensity of infection 85.06 percent at 15 DAS with 2.1×10^7 cfu/g was recorded by *Alternaria eichhorniae* @ 2 percent, followed by *Cercospora rodmanii* being 75.20 percent at 15 DAS with 1×10^6 - 10^8 cfu/g @ 2 percent and *Fusarium pallidoroseum* 62.20 percent at 15 DAS with 3.0×10^6 cfu/g @ 2 percent. At 7 DAS, range was between 76.30 to 46.10 percent of all fungi tested by formulated product. And on 11 DAS after spraying the range was 79.40 to 52.06 percent. *Alternaria eichhorniae* formulated product was virulent and significantly better than other fungi product. All formulated product were tested with chemical weedicide whose range was between 79.60 to 90.33 percent. Since chemical herbicides are reported to increase specific plant diseases, and the mechanism of increased susceptibility has been elucidated as suppression of resistance responses (Altman and Campbell, 1977; Huber *et al.*, 2005; Mekwatanakan and Sivasithamparam, 1987) [1, 5, 7].

A talc based wettable powder formulation of biocontrol fungi is effective in retaining the spore viability and for getting maximum efficiency under field conditions (Burges, 1998) [2]. There are reports on the talc-based formulations of biocontrol agents (Walker and Connick, 1983) [16]; Krishnamurthy and Gnanamanickam, 1998) [6]. Praveena (2003) [10] developed a wettable powder formulation of *Fusarium pallidoroseum* containing 40 percent fungus, 2 percent each of talc, sucrose and glycerol and 54 percent talc. Application of this @ 5 g and 10 g/100 ml recorded 82.22 percent and 97.78 percent disease intensity on water hyacinth plants respectively. The prepared formulated product were kept at room temperature (25±2 °C), at 35 °C and in refrigerator (10 °C). Viability of the antagonist in each formulation was determined at one month interval up 4 months. In the present finding increased shelf life in

different formulations were observed in all products at room temperature (25±2 °C), at 35 °C and at refrigerator (10 °C). The formulated products were retained viability of spores up 4 months in refrigerator condition, at room temperature, product was retained viability of spores up to 2 months and at high temperature (35 °C), product was retained viability of spores up to 1 month only. After this period, a sudden decline in the spore/fungal propagules viability was observed. Earlier reports also indicated a negative correlation between the spore viability and storage period (Nagesh *et al.*, 2001, Praveena, 2003, Faizal, 1992) [8, 10, 4]. Faizal (1992) [4] noticed that in the wettable powder formulation of *F. pallidoroseum*, the spores retained 75 percent viability for four weeks and thereafter there is a marked decrease in the virulence of the fungus. Praveena (2003) [10] reported that in the wettable powder formulation of *F. pallidoroseum*, from the initial spore count of 8.07×10^8 cfu/g, it was decreased to 6.39×10^7 cfu/g within 1 MAS and it reduced drastically to 7.0×10^4 cfu/g by 5 MAS.

According to TeBeest and Templeton (1985) [15], an effective organism for biological weed control should possess several key characteristics. It should be easy to culture in laboratory or industrial settings to facilitate mass production. The organism must produce infective structures, such as mycelia or spores, that are capable of initiating disease in target weed species. These structures should exhibit high viability and a long shelf life to ensure their effectiveness over time and during storage. Additionally, the organism should be capable of establishing infections and completing its disease cycle under a wide range of environmental conditions, including varying temperatures, humidity levels, and soil types. This ensures consistent performance in diverse agroecological zones. The biocontrol agent should also be cost-effective to produce and apply, making it economically viable for large-scale agricultural use. Furthermore, it must provide a high and predictable level of weed control to be considered reliable. Importantly, the organism must be safe for users, non-target organisms, and the environment, minimizing ecological and health risks.

Conclusion

Alternaria eichhorniae fulfils many of these criteria, demonstrating strong potential as a mycoherbicide, followed by *Cercospora rodmanii*, and *Fusarium pallidoroseum* in improving weed suppression across various environmental conditions. These formulations could be especially valuable for large-scale applications in diverse agricultural ecosystems, supporting sustainable and environmentally friendly weed management practices. Situations in which environment quality are restored in both ecologically sound farming systems and native habitats will benefit from the use of effective bioherbicides.

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