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Evaluation of mycoherbicide formulation for the management of *Parthenium*

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Abstract
The fungus *Curvularia lunata* is being developed as a mycoherbicide to control *Parthenium hysterophorus* L. 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 (3 months old). Talc-based formulations of *C. lunata* 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 *Parthenium* plants. Reducing the talc powder in the dust formulation from 50% to 10% did not affect efficacy. The most virulent fungi *C. lunata* showed a damage intensity of 85.64% with 2.85×106 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, *C. lunata,* biological control, PDA, concentration

Introduction
Parthenium hysterophorus L., known as congress grass, is a highly invasive weed from the *Asteraceae* family. Thriving in varied environments like fields, roadsides, and lawns, it spreads rapidly due to prolific seed production, environmental tolerance, and phytotoxic effects on other plants. Native to tropical America, it was introduced to India via Mexican wheat imports under the USA PL 480 scheme and now infests over 35 million hectares. Although officially reported in the 1950s, records hint at its presence as early as 1814. *Parthenium* has become a major agricultural problem, causing severe yield losses up to 97% in Ethiopian sorghum and major reductions in rice, tomato, and sorghum yields in India. It aggressively competes for resources, hosts pests and diseases, suppresses surrounding vegetation through allelopathy, and disrupts nitrogen fixation. Its massive pollen production further accelerates its spread. Beyond agriculture, it poses significant health risks, causing dermatitis, respiratory issues, and accounting for 40% of plant-related dermatitis cases in India. The economic burden includes management and healthcare costs totaling millions annually. Biological control using insects, pathogens, and competitive plants is now considered a sustainable and eco-friendly strategy for managing this invasive weed where chemical control is ineffective or uneconomical.

Materials and Methods
Preparation of talc-based bioherbicide formulation
A talc-based bioformulation of the fungi (*Alternaria alternata*, *Colletotrichum gloeosporioides* and *Curvularia lunata*) was prepared, as all these have maximum intensity of infection.

For the preparation of inoculum, pure culture of *Alternaria alternata*, *Colletotrichum gloeosporioides* and *Curvularia lunata* were maintained on PDA discs of 5mm diameter from seven-day old culture of *A. alternata*, *C. gloeosporioides* and *C. lunata* were inoculated into 100 ml sterilized potato dextrose 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: fungal propagules (spores/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±2 °C) and under refrigeration (4 °C) (Naseema *et al*., 2004) [9].

Evaluation of bioherbicide formulation for management of *Parthenium*

Different talc-based bioherbicide formulations and chemical weedicide were evaluated for the management of *Parthenium*.

Treatment details

Treatment:-5 (Five)

T_1 *Alternaria alternata* mycoherbicide formulation @ 2 percent

T_2 *Colletotrichum gloeosporioides* mycoherbicide formulation @ 2 percent,

T_3 *Curvularia lunata* mycoherbicide formulation @ 2 percent

T_4 Chemical weedicide (glyphosate 41% SL) @ 0.50 percent

T_5 control

Replication: 4 Design: CRD

Parthenium 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 bioherbicide formulations were spray inoculated on transplanted *Parthenium* plants using a hand automizer sprayer @ 2 percent concentration for bioherbicide formulation and 0.50 percent for chemical weedicide glyphosate. *Parthenium* 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 and 15 DAS (Days after spray) and PDI was calculated using the formula given by (Wheeler 1969) [14].

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

Table 1: Disease rating scale

Grade/Score	Description
0	No symptoms/ Mild symptoms
1	1-10 percent of the leaf area is infected
2	11-20 percent of the leaf area is infected
3	21-30 percent of the leaf area is infected
4	31-40 percent of the leaf area is infected
5	41-50 percent of the leaf area is infected
6	51-60 percent of the leaf area is infected
7	61-75 percent of the leaf area is infected
8	>75 percent of the leaf area is infected
9	Complete drying of plant

To study the shelf life of bio-formulations

In order to find out the shelf life of talc-based bioformulation, 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 15 days intervals by enumerating the number of colony forming units (cfu) in one-gram of talc-based bioherbicide formulation by serial dilution technique (Timonin, 1940) [12].

Experimental result

Development of talc-based bioherbicide formulations

Bio herbicide formulations of three most virulent fungi were prepared by following the method and composition given by Naseema *et al*., (2004) [9] *i.e*., fungal propagules (spores/ mycelium)-40 g, talc-54 g, sucrose-2 g, tween 80-2 g and glycerol-2 g. After proper blending, standard cfu/g of *Alternaria alternata*, *Colletotrichum gloeosporioides* and *Curvularia lunata* were maintained 1.0×106 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 on *Parthenium*. The disease intensity (PDI) was recorded at 7 DAS and 15 DAS (Table 1, Plate 1).

Among the formulations, maximum disease intensity (72.60%) at 7 DAS and 85.64 percent at 15 DAS was caused by *Curvularia lunata*, followed by *Alternaria alternata* with PDI

66.80 percent at 7 DAS and 77.70 percent at 15 DAS and minimum disease intensity (57.80%) at 7 DAS and 70.70 percent at 15 DAS by *Colletotrichum gloeosporioides* (Table 1, Plate 1).

Chemical weedicide (glyphosate) @ 0.50 percent caused 100 percent damage at 7 DAS (Table 1, Plate 1). However, *Curvularia lunata* talc-based mycoherbicide formulation was found significantly effective compared to other bio-formulations and next to chemical weedicide.

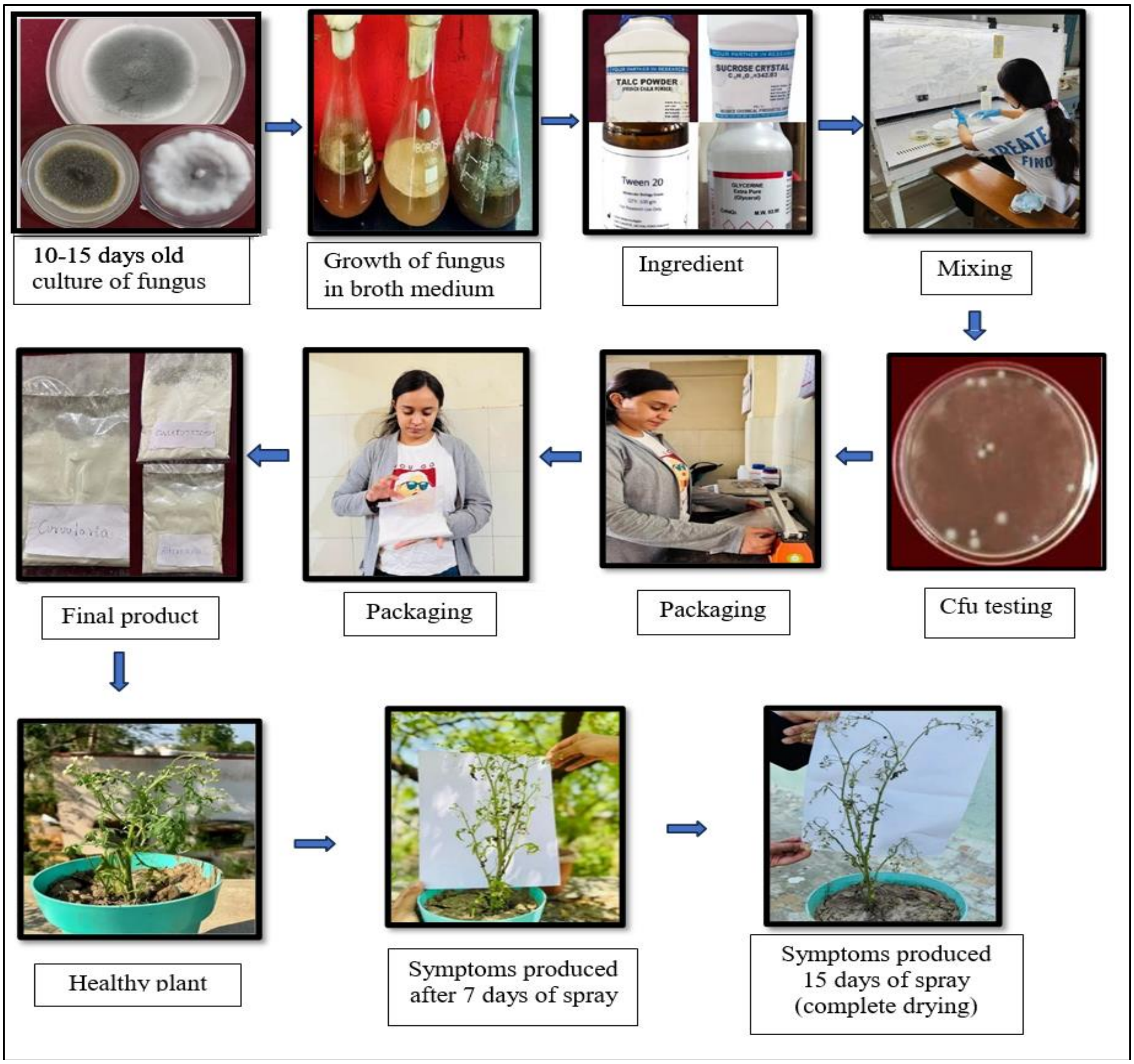

Plate 1: Preparation and evaluation of mycoherbicide formulation for the management of *Parthenium*

Table 1: Effect of different mycoherbicide formulations on *Parthenium*

S. No.	Formulations	Dose	*Disease intensity on *Parthenium* (%)	
			7 DAS	15 DAS
1	*Curvularia lunata*	2.0%	72.60 (58.44)	85.64 (67.26)
2	*Alternaria alternata*	2.0%	66.80 (54.97)	77.70 (61.91)
3	*Colletotrichum gloeosporioides*	2.0%	57.80 (49.56)	70.70(57.08)
4	Chemical weedicide (glyphosate)	0.50%	100 (90.00)	100 (90.00)
5	Control (water spray)	-	0	0
SEm±			0.64	0.72
C.D. at 5%			1.97	2.17
C.V.%			2.66	2.95

Note: Average of three replications; Figures in parentheses are arcsine √ percent angular transformed values. DAS = Days after spray

Studies on shelf life of mycoherbicide formulations

The talc-based mycoherbicide formulations of *Curvularia lunata* and *Alternaria alternata* 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 three months. Viability of the fungal propagules were tested at 15 days 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 *C. lunata* bio-formulations at all storage temperature was 2.85×106 per gram. At 10 °C viability of spores/fungal propagules were 2.33×106 cfu/g in 1-month of storage, 1.88×106 cfu/g in 2-month of storage, 1.10×104 cfu/g in 3-month of storage. So, that product stored at 10 °C have no extreme decline in cfu/g. However, at 25±2 °C viability of *C. lunata* propagules were decreased from 2.85×106 cfu/g to 0 cfu/g in 3-month of storage, 1.77×106 cfu/g in 1-month, 6.42×105 cfu/g in 2-month and 0 cfu/g in 3-month. Although at 35 °C gradual reduction in the viability of propagules was reduced to 4.51×104 cfu/g in 1-month, thereafter, a sudden decline in the viability was recorded to 0×106 cfu/g in 3-month (Table 2, Plate 2).

The viability of *Alternaria alternata* propagules were decreased from 2.38×106 cfu/g to 6.84×104 cfu/g at 10 °C at three months of storage. While at 25±2 °C viability of spores/ fungal propagules initially 2.38×106 cfu/g but gradually decreased from 1-month of storage 1.03×106 cfu/g to 5.05×104 cfu/g in 2-month and in 3 month it was recorded 0 cfu/g. At the temperature 35 °C, initially cfu/g was 2.38×106 and declined to 0 cfu/g at 3 months of storage (Plate 2).

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

Sr. No.	Days		*Curvularia lunata*			*Alternaria alternata*	
		10 °C	25±2 °C	35 °C	10 °C	25±2 °C	35 °C
1	0 (Initial)	2.85 x 106	2.85 x 106	2.85 x 106	2.38 x 106	2.38 x 106	2.38 x 106
2	15	2.65 x 106	2.22 x 106	7.75 x 105	2.11 x 106	1.88 x 106	4.44 x 105
3	30	2.33 x 106	1.77 x 106	6.51 x 104	1.89 x 106	1.03 x 106	6.3 x 104
4	45	2.04 x106	9.33 x 105	0	1.47 x 106	7.53 x 105	0
5	60	1.88 x106	6.42 x 105	0	1.10 x 106	5.05 x 104	0
6	75	2.2 x 105	1.31 x 104	0	1.87 x 105	0	0
7	90	1.5 x 104	0	0	1.10 x 104	0	0

***Average of three replications**

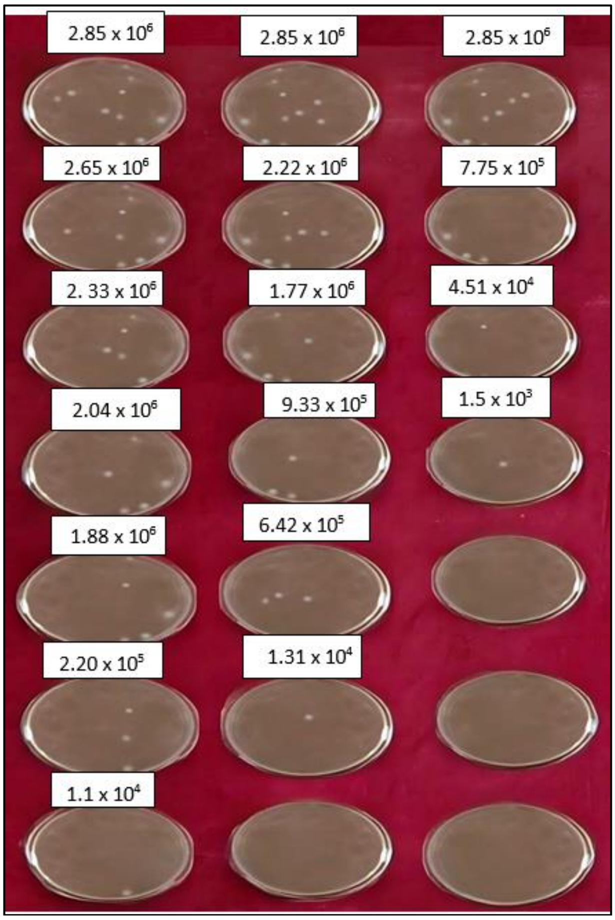

Plate 2: Shelf life of *Curvularia lunata* mycoherbicide formulation stored at different temperature

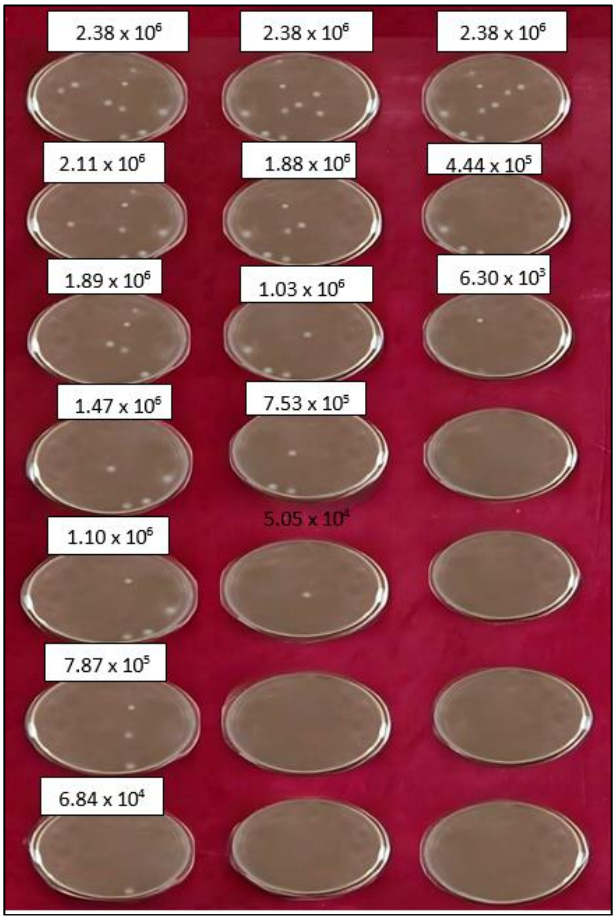

Plate 3: Shelf life of *Alternaria alternata* mycoherbicide formulation stored at different temperature

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 bioherbicides 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 formulation of three fungal pathogens was developed to evaluate their bio-herbicidal potential against *Parthenium hysterophorus*. Mycelial biomass (400 g) from broth cultures was homogenized and incorporated into 20 g of Tween-80, 20 g glycerol, 20 g sucrose and 540 g sterilized talc powder and air dried (Naseema *et al.* 2004) [9]. The formulations were applied to healthy *Parthenium* plants through foliar spray (2%). *Curvularia lunata* bioformulation exhibited the highest disease intensity (72.60%) at 7 DAI and 85.64% at 15 DAI, followed by *Alternaria alternata* (66.80% and 77.70%, respectively), indicating strong pathogenic efficacy. *Colletotrichum gloeosporioides* showed moderate effectiveness with 57.80% and 70.70% disease intensity. The chemical herbicide glyphosate 41 SL @ 0.5% achieved complete control at both intervals. Among the bio-herbicidal agents, *Curvularia lunata* and *A. alternata* proved most effective, prompting further studies on their shelf life and viability under different storage conditions. Spore viability studies over 90 days revealed that lower temperatures (10 °C) significantly preserved fungal viability for both pathogens. For *Curvularia lunata*, spore counts remained relatively stable at 10 °C but declined sharply at 25±2 °C and were entirely lost by 60 days at 35 °C. Similarly, *A. alternata* showed a gradual reduction in viability, with complete loss at 35 °C after 45 days and minimal survival at 10 °C through 90 days. These findings confirm that both fungal agents are effective bio-herbicides against *Parthenium*, with better long-term storage potential at refrigerator and room temperature conditions.

Similar findings have been made by Boyette *et al.* (1993) [2] reported that granular formulations of *Fusarium oxysporum* (such as Pesta) were more effective for weed control than liquid formulations. Klein *et al.* (1995) [7] found that drying *Colletotrichum gloeosporioides* spores in kaolin with vegetable/mineral oil enhanced their herbicidal efficacy. Awadhiya and Sharma (1997) [1] achieved maximum seedling mortality using *Sclerotium rolfsii* formulations under high humidity and moderate temperatures. Kauraw *et al.* (1997) [5] reported that *Fusarium pallidoroseum* significantly inhibited the germination and growth of *Parthenium hysterophorus*, suggesting its potential as a host-specific biocontrol agent. Zhang and Watson (1997) found that *Exserohilum monoceros* caused high mortality in *Echinochloa* spp. when applied as a dry powder or oil emulsion. Smitha (2000) [11] prepared a talc-based formulation of *Trichoderma longibrachiatum* using sterilized talc and carboxymethyl cellulose. Kalam *et al.* (2014) [6] evaluated *Phoma herbarum* FGCC#54 metabolites, noting high phytotoxicity with formulations containing surfactants like Tween-80. Walker and Connick (1993) [2] demonstrated that a sodium alginate and clay formulation of *Alternaria crassa* could be stored at 4 °C for 6 to 8 months. Connick *et al.* (1996) [3] showed improved shelf life of *Colletotrichum truncatum* conidia when formulated in wheat flour-kaolin granules. Nakkeeran *et al.* (1997) [8] observed a decline in *Trichoderma* population from 31×10^6 to 13×10^6 cfu/g over 75 days in talc-based formulations stored between 20 °C and 30 °C. Similarly, Honeycutt *et al.* (2001) [4] observed 68% reduction in binucleate *Rhizoctonia* spp. propagules after six months in pesta and rice flour formulations, with the greatest loss within the first 2 months. *Paecilomyces lilacinus* spores stored at 0 °C with tapioca broth-talc or paddy grain-talc formulations retained over 80% viability after 150 days. Praveena (2003) [10] highlighted that *F. pallidoroseum* dust formulations rapidly lost viability at room temperature, while refrigerated wettable powder formulations retained viability up to four months.

According to TeBeest and Templeton (1985) [13], 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

Curvularia lunata fulfils many of these criteria, demonstrating strong potential as a mycoherbicide, followed by *Alternaria alternata* and *Colletotrichum gloeosporioides* 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.

References

1. Awadhiya GK, Sharma ND. Possible control of *Parthenium hysterophorus* L. by the use of fungal pathogen. Journal of Myco-pathological Research. 1997;35:51-53.
2. Boyette CD, Abbas HK, Connick WJ. Evaluation of *Fusarium oxysporum* as a potential bioherbicide for sicklepod (*Cassia obtusifolia*), coffee senna (*C. occidentalis*), and hemp sesbania (*Sesbania exaltata*). Weed Science. 1993;41(4):678-681.
3. Connick WJ, Daigle DJ, Boyette CD, Williams KS, Vinyard BT, Quimby PC. Water activity and other factors that affect the viability of *Colletotrichum truncatum* conidia in wheat flour-kaolin granules

(Pesta). Biocontrol Science and Technology. 1996;6(2):277-284.
4. Honeycutt EW, Benson DM. Formulation of binucleate *Rhizoctonia* spp. and biocontrol of *Rhizoctonia solani* on impatiens. Plant Disease. 2001;85(12):1241-1248.
5. Kauraw LP, Chile A, Bhan VM. Evaluation of *Fusarium pallidoroseum* (Cooke) Sacc. for the biocontrol of *Parthenium hysterophorus* L. Journal of Myco-pathological Research. 1997;35:70-74.
6. Kalam S, Pandey A, Pandey AK. Mass production and formulation of herbicidal metabolites from *Phoma herbarum* FGCC#54 for management of some prominent weeds of Central India. American International Journal of Research in Formal, Applied and Natural Sciences. 2014;5(1):40-47.
7. Klein TA, Auld BA, Fang W. Evaluation of oil suspension emulsions of *Colletotrichum orbiculare* as a mycoherbicide in field trials. Crop Protection. 1995;14(3):193-197.
8. Nakkeeran S, Sankar P, Jayarajan R. Standardization of storage conditions to increase the shelf life of *Trichoderma* formulations. Journal of Mycology and Plant Pathology. 1997;27(1):60-63.
9. Naseema A, Praveena R, Ancy Salim M. Ecofriendly management of water hyacinth with a mycoherbicide and cashew nut shell liquid. Pakistan Journal of Weed Science Research. 2004;10(1-2):93-104.
10. Praveena R. Management of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] using fungal pathogens [Ph.D. thesis]. Thrissur: Kerala Agricultural University; 2003. p. 222.
11. Smitha KP. Management of foliar blight of amaranthus [*Amaranthus tricolor* L.] caused by *Rhizoctonia solani* Kühn using microbial antagonists [M.Sc. thesis]. Thrissur: Kerala Agricultural University; 2000. p. 72.
12. Timonin MI. The interaction of higher plant and soil microorganisms. Microbial population of seedlings of certain cultivated plants. Canadian Journal of Research. 1940;18(7):307-317.
13. TeBeest DO, Templeton GE. Mycoherbicide progress in the biological control of weeds. Plant Disease. 1985;69:6-10.
14. Wheeler BEJ. An introduction of plant diseases. London: John Wiley & Sons Ltd.; 1969. p. 301.
15. Zhang W, Watson AK. Efficacy of *Exserohilum monoceras* for the control of *Echinochloa* spp. in rice (*Oryza sativa*). Weed Science. 1997;45(1):144-150.