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Screening of Effective Formulants for *Fusarium* sp. FGCCW#16 Cell free Culture broth in Controlling *Parthenium hysterophorus*

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ABSTRACT

Natural herbicidal compound from *Fusarium* sp. FGCCW#16 cell free culture broth was used as a mycoherbicide product for the control of *Parthenium hysterophorus* a weed that causes considerable problem to the biodiversity, agriculture, economy, and health of livestock and human beings. Formulations containing distilled water or culture filtrate and different adjuvants (different oil like mineral oil, palm oil or soybean oil), surfactant like Glycerol and Tween 80) were evaluated in order to increase the bioherbicidal activity through post-emergence bioassays. The herbicidal activity of culture filtrate was improved using different combinations of adjuvants. The best formulation was 5% (w/v) of mineral oil, 5% (w/v) of surfactant (glycerol and Tween 80) and a hydrophilic-lipophilic balance (HLB), which resulted in a higher herbicidal activity (100%) (complete death of plants). The suitable combination of adjuvants in association with culture filtrate from *Fusarium* sp. FGCCW#16 increased up to 2.5 times the efficiency of bioherbicide for the post-emergence control of *Parthenium hysterophorus* weed.

Keywords: Noxious invasive weed; Parthenium; FGCCW#16; Mycoherbicide; Mass Production; Formulation

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1. INTRODUCTION

Parthenium hysterophorus, enlisted in Global Invasive Species database (Callway et al. 2004) Thus, nowadays it is found infesting almost all parts of the world. Its rapid and extensive spread can be attributed to both human activities during globalization and colonizing potential of the weed plant over wide range of habitats and climatic conditions. Parthenium weed is found in both natural and agroecosystems. It shows many adverse effects on agriculture, biodiversity, and health of animals and human beings. The control of Parthenium sp. is usually accomplished through the use of chemical herbicides such as glyphosate. However, over the years, some species, including Parthenium sp., become resistant to the mechanism of action of certain chemicals (Bracamonate et al. 2016). Such synthetic products, like glyphosate, effectively assist the farmer in achieving high productivity in short-term. However, in the long-term, they have negative results for the society and environment, thus requiring studies for using natural bioproducts (Bonny 2016; Confortin et al. 2019; Todero et al. 2019; Balesteros et al. 2014; Keerthi et al. 2019; Reichert et al. 2019). Taking into account this scenario, the prospection and discovery of new molecules appear as an important tool for the control of resistant weeds (Hassan et al. 2020). Some studies have shown promising results in the weed control using fermented broth containing the secondary metabolites produced by fungi via submerged fermentation (Brun et al. 2016; de Souza et al. 2015; de Souza et al. 2017; Triolet et al. 2020; Kaur and Aggarwal 2015). Secondary metabolites can damage weeds by penetrating the plant followed by the destruction of the cell wall and induction of necrotic lesions (Kumar et al. 2017; Varejao et al. 2013).

On the other hand, many promising biomolecules are early discarded during the stages of mycoherbicide development because they present low herbicidal activity. In a general way, low efficiency is a consequence of the very low concentration of biomolecules in the fermentation media (Boyette et al. 2016). Therefore, some strategies to concentrate these molecules are essential to obtain an efficient product. The formulation is an important parameter for successful product development. The word formulation is described as a mixture of an active ingredient (a.i.) and the appropriate or compatible inert ingredients. Adjuvants are the most popular inert ingredients commonly found in bioherbicide formulations. Adjuvants are well known to improve the efficacy of bioherbicides by altering their physicochemical properties. Surfactants, emulsifiers, and hydrophilic polymers are examples of adjuvants important for improving the effectiveness of bioherbicides. Mycoherbicide compounds are generally applied to weeds in the form of an emulsion, which can increase weed control stability and effectiveness (Castro et al. 2013). The hydrophilic polymers are readily miscible with water; emulsifiers are employed to blend water (hydrophilic) and oil (hydrophobic) components in a formulation into a stable emulsion. Unfortunately, the cost of bioherbicides sometimes increases because of the high cost of the adjuvants used in the formulation. Additionally, a careful selection of surfactants must be taken into consideration, since certain ingredients used in herbicide formulations can be toxic to humans (Hazrati et al. 2017). The vegetable oil and surfactants increased the adsorption ability of polar molecules, dissolved cuticular fatty acids, and thereby improved the penetration of hydrophilic active substances (Lost and Raetano 2010).

In the meanwhile, it is necessary to use an adequate combination of adjuvants in the formulation to increase the herbicidal activity. Adjuvants are substances present in a formulation with the aim of modifying the biological activity or the application characteristics of the formulation (Mirgorodskaya et al. 2020; De Almedia et al. 2020; Bastos et al. 2017). This is highlighted in a study reported by Bastos et al. (2017), in which the authors increased three times the herbicidal activity of culture filtrate from *Diaphorte* sp. obtained by solid state fermentation using 5 wt % of palm oil , 5 wt % of surfants and HLB 15.0.

The genus *Fusarium* sp. has been widely used in the production of metabolites for weed control (Reveglia et al. 2018). The herbicidal activity of different species (*Fusarium avenaceum, F. acuminatum, F. redolens, F. culmorum*/*F. cerealis* and *F. solani*) was evaluated in different grass species, demonstrating promising results using *F. avenaceum* and *F. acuminatum* (Pearson et al. 2016). Previously, isolated and identified the fungus *Fusarium* sp. FGCCW#16 was obtained from Parthenium weed with herbicidal activity towards target plants (Singh and Pandey, 2019). Based on these aspects, the objective of this study was to evaluate the efficiency of using adjuvants in association with fermented broth produced by the fungus *Fusarium* sp. FGCCW#16 isolated from the Parthenium, for the control of *Parthenium* weeds.

2. MATERIALS AND METHODS

2.1. Materials:

Potato dextrose agar (PDA), $(NH_4)_2SO_4$, $FeSO_4.7H_2O$, $MnSO_4.H_2O$, $MgSO_4$, Tween 80, Corn steep liquor (CSL), Palm oil (*Elaeis guineensis*), Soybean oil, Sucrose, Mineral oil and Glycerol were used in experiment.

2.2. Fermentation:

Fusarium sp. FGCCW#16 was previously isolated by Singh and Pandey (2019). The culture was maintained in a petri dish with PDA medium between 4 °C and 6 °C and sub cultured every 15 days. For pre-inoculum cell production was done incubating the culture on PDA in a Petri dish for 8 days at 28 ºC. Afterward, three discs of 6 mm of fungal mycelium were transferred to the fermentation flasks. The fermentation was carried out in Erlenmeyer flasks containing 125 mL of fermentation medium under stirring in an Orbital Incubator Shaker at 31°C and 200 rpm for 7 days. The medium was composed of (g/L): Sucrose (10.0), corn steep liquor (50.0), $(NH_4)_2SO_4$ (0.5), $FeSO_4.7H_2O$ (1.0), $MnSO_4.H_2O$ (1.0) and $MgSO_4$ (0.5). The initial pH was adjusted to 6.0 (Brun et al. 2016). After the fermentation, the cells were separated from the medium by centrifugation at 4000 rpm for 10 min and the supernatant was filtered using a 0.45µm Polyvinylidene Difluoride (PVDF) membrane. The filtered samples (culture filtrate) were used to formulate the mycoherbicide applied in the control of Parthenium weed.

2.3. Formulation of Mycoherbicide:

Initially, three different oils (palm, soybean, and mineral oil) in association with distilled water or culture filtrate were tested (Table 1). The objective of using oil in the formulation was to increase the adhesion and permanence of the fermented in the foliar area of the plants (Todero et al. 2018).

The formulations were prepared at 25ºC using a homogenizer. Firstly, oil (oil and glycerol) and aqueous (distilled water or culture filtrate and Tween 80) phases were homogenized separately for 1 min at 7000 rpm. Then, the oil phase was slowly added to the aqueous phase and the mixture was homogenized at 7000 rpm for 10 min. The final emulsion volume was 100 mL. The samples were maintained at rest for 1 h before their use in the bioassays.

2.4. Bioassays:

The formulation efficiency in the control of *Parthenium* sp. was evaluated through post-emergence bioassays with young plants. The plants were collected in the experimental area of R. D. University, Jabalpur and transplanted into polyethylene cups containing 200 g of the sterilized soil. They were cultivated in a greenhouse for 15 days before the application of mycoherbicide, which was applied using a hand sprayer. Phytotoxicity assays were also performed with the respective formulations, however replacing the culture filtrate by distilled water. The plant injury was visually estimated 15 days after mycoherbicide application, following the methodology proposed by Frans and Crowley (Frans et al. 1986).

3. RESULTS AND DISCUSSION

The influence of oil used in the formulations is presented in Table 1. Treatments T1 and T2 formulated with palm and soybean oil, respectively, had a slight effect on the aerial part of Conyza sp. plants, whereas the treatment T3, formulated with mineral oil, showed a severe effect. The control treatments T4 and T5 did not present a phytotoxic effect, while the control treatment T6 showed a slight effect on plants. The treatment T7, composed of culture filtrate, had a low effect on plants, with only a slight yellowing at the leaf ends. Among the oils tested in the study, mineral oil presented the best results. Therefore, it was selected for the next steps of processing and analysis.

Table 1: Bioherbicidal activity of culture broth formulations with different oils

Treatment	Formulation (ml)	Herbicidal activity (%)
T1	Culture filtrate (95)+ Palm oil (5)	10
T2	Culture filtrate (95)+ Soyabean oil (5)	10
T3	Culture filtrate (95)+ Mineral oil (5)	90
T4	Distilled water (95) + Palm oil (5)	5
T5	Distilled water (95) + Soyabean oil (5)	5
T6	Distilled water (95) + Mineral oil (5)	10
T7	Culture filtrate (100)	5
T8	Distilled water (100)	0

The obtained results suggest that the mycoherbicide produced by *Fusarium* sp. FGCCW#16 could be a hydrophilic molecule because surfactants with a high HLB increase the cuticle hydration. Consequently, it promotes a better permeability of hydrophilic herbicides into the leaves, increasing their diffusion rate at a constant concentration gradient (Hess and Foy, 2000). The results obtained were similar to those obtained by Bastos et al. (2017), whose authors verified that the efficiency of formulated bioherbicide from *Diaporthe* sp. was higher with HLB 15.0.

The formulation is an important step in the development of a mycoherbicide with metabolites from fungi. Without an adequate combination of adjuvants, many bioproducts with great attractiveness in the market are discharged. In this work, the efficiency of the mycoherbicide was improved approximately 2.5 times when compared with non-formulated products. Bastos et al. (2017) [24] increased 3 times the efficiency of a bioherbicide produced from *Diaphorte* sp. Likewise, Pes et al. (2016) accentuated the suppressive effect on the growth of *Conyza* sp. and *Echinochloa* sp. for a bioherbicide formulated from *Diaphorte* sp.

Aybeke(2017) reported toxic effects of *Fusarium oxysporum* on a root parasitic weed (*Orobanche* spp.). Li et al. (2014) reported that ethyl acetate extract of the fermentation broth of *Fusarium proliferatum* provided selective phytotoxic activity against the radicle growth of *Amaranthus retroflexus*. Nevertheless, there are no reports about metabolites from *Fusarium* sp. in the control of Parthenium sp. up to now. Therefore, this work can be considered the first study related to the herbicidal activity of metabolites from *Fusarium* sp. FGCCW#16 isolated from Jabalpur in an infected Parthenium for the control of this weed.

4. CONCLUSIONS

In this study, the necessity of a correct combination of adjuvants to increase the herbicide activity of culture filtrate was demonstrated. Depending on the adjuvant's combination, the herbicidal activity may reach values insufficient to follow the next steps in the development stage. The better result of herbicidal activity was obtained with a formulation containing 5% (w/v) of mineral oil, 5% (w/v) of surfactant and HLB of 15, a condition in which a complete death of *Parthenium* sp. plants was observed. This promising combination of adjuvants and culture filtrate from *Fusarium* sp. FGCCW#16 increased 2.5 times the efficiency of mycoherbicide for the post-emergence control of *Parthenium* sp.

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6. REFERENCES

Aybeke, M. *Fusarium* Infection Causes Genotoxic Disorders and Antioxidant-Based Damages in *Orobanche* spp. *Microbiological Research*, 2017, 201, 46-51.

Balesteros, M.R.; de Sá, L.R.V.; Pereira, P.M.; da Silva, M.; de Oliveira, M.A.L.; Ferreira-Leitão, V.S. Monitoring of Atrazine Biodegradation by *Pleurotus ostreatus* INCQS 40310 Through the Simultaneous Analysis of Atrazine and its Derivatives by HPLC. *Biocatalysis and Biotransformation*, 2014, 32, 23-33.

Bastos, B.d.O.; Deobald, G.A.; Brun, T.; Dal Prá, V.; Junges, E.; Kuhn, R.C.; Pinto, A.K.; Mazutti, M.A. Solid-state Fermentation For Production of A Bioherbicide from *Diaporthe* sp. and Its Formulation to Enhance the Efficacy. 3 *Biotech*, 2017, 7, 135-143, https://doi.org/10.1007/s13205-017-0751-4.

Bonny, S. Genetically Modified Herbicide-Tolerant Crops, Weeds, and Herbicides: Overview and Impact. Environmental Management, 2016, 57, 31-48

Boyette, C.D.; Hoagland, R.E.; Stetina, K.C. Efficacy Improvement of a Bioherbicidal Fungus Using a Formulation-Based Approach. *Am. J. Plant Sci*., 2016,7:2349–2358.

Bracamonte, E.; Fernández-Moreno, P.T.; Barro, F.; De Prado, R. Glyphosate-Resistant *Parthenium hysterophorus* in the Caribbean Islands: Non-Target Site Resistance and Target Site Resistance in Relation to Resistance Levels. *Front Plant Sci*. 2016, 6, 7, 1845.

Brun, T.; Rabuske, J.E.; Todero, I.; Almeida, T.C.; Junior, J.J.D.; Ariotti, G.; Confortin, T.; Arnemann, J.A.; Kuhn, R.C.; Guedes, J.V.C.; Mazutti, M.A. Production Of Bioherbicide by *Phoma* sp. In A Stirred-Tank Bioreactor. *3 Biotech*, 2016, 6, 230-238.

Callaway, R. A.; Ridenour, W.M. "Novel weapons: invasive success and the evolution of increased competitive ability," *Frontiers in Ecology and the Environment*2004,2 8,436–443.

Castro, M.J.; Ojeda, C.; Cirelli, A.F. Green Materials for Energy, Products and Depollution. Springer; Dordrecht, Germany:. *Surfactants In Agriculture*, 2013,287–334.

Confortin, T.C.; Todero, I.; Soares, J.F.; Luft, L.; Brun, T.; Rabuske, J.E.; Nogueira, C.U.; Mazutti, M.A.; Zabot, G.L.; Tres, M.V. Extracts from Lupinus albescens: Antioxidant Power and Antifungal Activity In vitro Against Phytopathogenic Fungi. Environmental Technology, 2019, 40, 1668-1675.

De Almeida, T. C.; Klaic, R.; Ariotti, G.; Sallet, D.; Spannemberg S.S.; Schmaltz, S.; Foletto E. L.; Kuhn R.C.; Hoffmann, R.; Mazutti, M.A. Production and Formulation of A Bioherbicide

As Environment-Friendly And Safer Alternative for Weed Control. *Biointerface Res. Appl. Chem*., 2020, 10, 5938-5943.

de Souza, A.R.C.; Baldoni, D.B.; Lima, J.; Porto, V.; Marcuz, C.; Ferraz, R.C.; Kuhn, R.C.; Jacques, R.J.S.; Guedes, J.V.C.; Mazutti, M.A. Bioherbicide Production by *Diaporthe* sp. Isolated from The Brazilian Pampa Biome. *Biocatalysis and Agricultural Biotechnology*, 2015, 4, 575-578.

de Souza, A.R.C.D.; Baldoni, D.B.; Lima, J.; Porto, V.; Marcuz, C.; Machado, C.; Ferraz, R.C.; Kuhn, R.C.; Jacques, R.J.S.; Guedes, J.V.C.; Mazutti, M.A. Selection, Isolation, and Identification of Fungi for Bioherbicide Production. *Brazilian Journal of Microbiology*, 2017, 48, 101-108.

Frans, R.; Talbert, R.; Marx, D.; Crowley, H. Research Methods in Weed Science. 3rd ed.; Champaign: Southern Weed Science Society, United States, 1986, 29-46.

Hassan, A.; Ahmad, W.; Israr, M. Evaluation of Allelopathic Effect of *Carthamus ozyacantha* Against Wheat and Maize Seed Germination. *Lett Appl Nano Bio Science*, 2020, 9, 814-818.

Hazrati, H.; Saharkhiz, M.J.; Niakousari, M.; Moein, M. Natural Herbicide Activity of *Satureja hortensis* L. Essential Oil Nanoemulsion on the Seed Germination and Morphophysiological Features of Two Important Weed Species. Ecotoxicol. *Environ. Saf.* 2017,142,423–430.

Hess, F.D.; Foy, C.L. Interaction of Surfactants with Plant Cuticles. *Weed Technol*, 2000, 14, 807-813.

Kaur, M.; Aggarwal, N.K. Comparative Evaluation of Phytotoxicity of *Alternaria macrospora* Isolates, Potential Biocontrol Agent against Parthenium Weed. *Res. J. Bot.,* 2015, 10, 14-21.

Keerthi, P.; Singh, M.; Bishnoi, A. Role of Biological Control of Weeds and Bioherbicides. In: Advances in Agronomy. 1st ed.; Rawat, A.K.; Tripathi, U.K. Eds.; AkiNik Publications: New Delhi, India, Volume 4, 2019; pp. 61-79.

Kumar, M.; Madhupriya; Rao, G.P. Molecular Characterization, Vector Identification and Sources of Phytoplasmas Associated With Brinjal Little Leaf Disease in India. *3 Biotech*, 2017, 7, 1-11.

Li, S.; Shao, M.W.; Lu, Y.H.; Kong, L.C.; Jiang, D.H.; Zhang, Y.L. Phytotoxic and Antibacterial Metabolites from *Fusarium proliferatum* ZS07 Isolated from the Gut of Long-horned Grasshoppers. *J Agric Food Chem*, 2014, 62, 8997-9001.

Lost, C.A.R.; Raetano, C.G. Dynamic Surface Tension and Contact Angle Of Water Solutions with Spray Surfactants In Artificial And Natural Surfaces. *Eng Agr*, 2010, 30, 670-680.

Mirgorodskaya, A.B.; Kushnazarova, R.A.; Lukashenko, S.S.; Nikitin, E.N.; Sinyashin, K.O.; Nesterova, L.M.; Zakharova, L.Y. Carbamate-Bearing Surfactants As Effective Adjuvants Promoted The Penetration of The Herbicide Into The Plant. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2020, 586.

Pearson, K.A.; Taylor, A.F.S.; Fuchs, R.M.E.; Woodward, S. Characterisation and Pathogenicity of *Fusarium* Taxa Isolated from Ragwort (*Jacobaea vulgaris*) Roots. *Fungal Ecology*, 2016, 20, 186-192.

Pes, M.P.; Mazutti, M.A.; Almeida, T.C.; Curioletti, L.E.; Melo, A.A.; Guedes, J.V.C.; Kuhn, R.C. Bioherbicide based on *Diaporthe* sp. Secondary Metabolites in the Control of Three Tough Weeds. *Afr. J. Agric. Res*., 2016, 11, 4242-4249.

Reichert Júnior, F.W.; Scariot, M.A.; Forte, C.T.; Pandolfi, L.; Dil, J.M.; Weirich, S.; Carezia, C.; Mulinari, J.; Mazutti, M.A.; Fongaro, G.; Galon, L.; Treichel, H.; Mossi, A.J. New Perspectives for Weeds Control Using Autochthonous Fungi with Selective Bioherbicide Potential. *Heliyon*, 2019, 5.

Reveglia, P.; Cinelli, T.; Cimmino, A.; Masi, M.; Evidente, A. The Main Phytotoxic Metabolite Produced by a Strain of *Fusarium oxysporum* Inducing Grapevine Plant Declining in Italy. *Nat. Prod. Res*. 2018, 32, 2398- 2407.

Singh, A. K.; Pandey, A. K. Standardization of Various Parameters for Mycoherbicidal Metabolites Production from *Fusarium* sp. FGCCW#16 for *Parthenium hysterophorus* Management. *Journal of Research in Weed Science*, 2019, 2(3), 203-215.

Todero, I.; Confortin, T.C.; Luft, L.; Brun, T.; Ugalde, G.A.; de Almeida, T.C.; Arnemann, J.A.; Zabot, G.L.; Mazutti, M.A. Formulation of a

Bioherbicide with Metabolites from *Phoma* sp. *Scientia Horticulturae*, 2018, 241, 285-292.

Todero, I.; Confortin, T.C.; Soares, J.F.; Brun, T.; Luft, L.; Rabuske, J.E.; Kuhn, R.C.; Tres, M.V.; Zabot, G.L.; Mazutti, M.A. Concentration of metabolites from *Phoma* sp. Using Microfiltration Membrane for Increasing Bioherbicidal Activity. *Environmental Technology*, 2019, 40, 2364-2372.

Triolet, M.; Guillemin, J.P.; Andre, O.; Steinberg, C. Fungal-based Bioherbicides for Weed Control: A Myth or A Reality? *Weed Research*, 2020, 60, 60-77.

Varejão, E.V.V.; Demuner, A.J.; Barbosa, L.C.A.; Barreto, R.W. the Search for New Natural Herbicides –Strategic Approaches For Discovering Fungal Phytotoxins. *Crop Protection*, 2013, 48, 41-50.