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Formulation and pathogenicity of a bioherbicide for wild poinsettia control

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Adequate formulation of bioproducts represents one of the most challenging aspects of bioproduct development. The incorporation of adjuvants with bioagents can positively influence product development. However, it is indispensable to evaluate the sensitivity of bioagents to these adjuvants. The aim of this study was to determine the toxicity of seven adjuvants at different concentrations to *Bipolaris yamadae* (*Bipolaris euphorbiae*) fungus, to select a product compatible with this phytopathogen for a wettable powder formulation and to evaluate the pathogenicity of the formulation against wild poinsettia (*Euphorbia heterophylla*). The powder fraction of the formulation was made up of 1% anti-wetting silicon dioxide mixed with *B. yamadae* conidia to a final concentration of 10^7 conidia.ml^{-1}. The aqueous fraction was composed of 0.1% Geropon T36 compatibilizer, 0.075% silicone, the dispersant, 0.1% Tween 80 or tensioactive and 0.5% PVP K30 or spreading agent. The incidence of disease was observed in 83.6% of the plants inoculated with the formulated fungus, which was 79.0% higher than that in the plants inoculated with the bioagent only. These findings strongly suggest that the new formulation successfully controls *Euphorbia heterophylla* and greatly increases the pathogenicity of the fungus.

Key words: *Bipolaris yamadae*, *Euphorbia heterophylla*, adjuvants, biological control, phytopathogenic fungi.

INTRODUCTION

Weed control plays an important role in the management of economically important crops (Green, 2014; Zhu et al., 2020). The spread of weeds with biotypes that areresistant to chemical herbicides, concerns about environmental issues and the necessity of reducing production costs are the main factors that drive the search for new weed control strategies (Caldwell et al., 2012). The use of specific phytopathogens as bioherbicides is a potential strategy for weed management due to its practicality and environmental safety.

One factor that limits the advancement of this kind of

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weed control is the difficulty of obtaining adequate formulations. This limitation is caused by the need to add compounds to increase the efficiency and stability of the biocontrol agent, allowing it to remain in the environment and increasing the possibility of reaching and attacking the target plants (Caldwell et al., 2012; Loureiro et al., 2003).

Adjuvants are commonly used in agriculture to increase the action of chemical pesticides. The incorporation of these adjuvants into biological formulations can positively influencethe performance of the formulations, especially fungal conidia formulations, by maintaining low levels of available water to prevent conidial germination during storage, promoting adhesion and uniform spreading, protecting the phytopathogenfrom UV radiation and retaining the pulverized droplets on the foliar surface (Wraight et al., 2001). In addition, the adjuvants are able to modify the morphology of the epicuticular wax or promote injury to the leaf tissue, favouring microbial action in harming weed growth and development (Greavesand MacQueen, 1990; Womack and Burge, 1993; Prasad, 1994; Falk et al., 1994). However, these adjuvants can be toxic to the phytopathogen, so it is advisable to evaluate their toxicity to these microorganisms (Wyss et al., 2004; Keswani et al., 2016).The fungus *Bipolaris yamadae* (*Bipolaris euphorbiae*) (Marin-Felix et al., 2017) is a host-specific phytopathogen of *Euphorbia heterophylla* L. (Euphorbiaceae), commonly known as wild poinsettia. It is an invasive weed of great economic importance for crops, especially soybean. *B. yamadae* can be used as a bioagent to control this noxious weed; however, to enhance *B. yamadae* efficacy and survival, the development of effective formulations with proper additives is necessary.The present study aimed:

(1) to evaluate the toxicity of certain adjuvants to the fungus *B. yamadae*,
(2) to select certain compatible additives for this phytopathogen to compose a wettable powder formulation and
(3) to evaluate the pathogenicity of the developed formulation on *E. heterophylla* under green house conditions.

MATERIALS AND METHODS

Fungal strain and solid-state cultivation

This study was performed using the FCAV#569 fungal strain of *B. yamadae*. The strain was grown in Petri dishes containing Pontecorvo minimal medium according to Pontecorvo et al. (1953); it was modified by supplementation with peptone at 2 $g.L^{-1}$ and with starch replaced with glucoseat10 $g.L^{-1}$ (Penariol et al., 2008). The Petri dishes were kept at 25 ± 0.5°C for 10 days. In addition, the strain was preserved by ultrafreezing at -80°C in 2% malt broth containing 10% glycerol. Cultivation in the solid medium was performed to obtain a significant amount of *B. yamadae* conidia. The growth substrate was composed of a mixture of soybean hulls and sorghum in 60:40 m/m. The mixture was distributed in 250 ml Erlenmeyer flasks and then sterilized by autoclaving for 20 min at 121°C. The flasks were inoculated with three mycelial discs of 8mm diameter taken from fungal colonies grown on Pontecorvo minimal medium. The flasks were incubatedunder the same conditions described above.After incubation, the colonized mixture was dried, and the conidia were extracted according to Machado et al. (2013).

Bipolaris yamadae compatibility with chemical adjuvants

The list of the adjuvants evaluated for the fungal formulation and their respective concentrations and functions are presented in Table 1. Predetermined quantities of the adjuvants (Table 1) were added to flasks containing liquefied Pontecorvo minimal medium (Pontecorvo et al., 1953), and then the medium was poured into Petri dishes measuring 90 × 15 mm. For the control treatment, adjuvants were notadded to the minimal medium. After solidification, one mycelial disk of 5 mm diameter of precultured fungi was transferred to the centre of the plates.

In order to determine the adjuvant toxicity profile to the fungus, the biological index (BI) model proposed by Rossi-Zalaf et al. (2008) was used. This model takes into consideration parameters such as vegetative growth, sporulation and germination. It was calculated according the formula:

$$BI = \frac{47[VG] + 43[SPO] + 10[GER]}{100}$$

In which:BI = biological index; VG = percentage of vegetative growth after 10 days of incubation compared to that in the control; SPO = percentage of sporulation after 10 days of incubation compared to that in the control; GER = percentage of conidial germination after 7h of incubation. The BI toxicological classification of the adjuvants was made using the scale described by Rossi-Zalaf et al. (2008), where BI values between 0 and 41 were considered toxic to the fungus; BI values between 42 and 66 were considered moderately toxic; and BI values above 66 were considered compatible with the fungus.

Vegetative growth (VG) was analyzed by measuring in mm two perpendicular diameters on the 10^{th} day of incubation. After this period, the conidia produced on the surface of the colony were removed by scraping and transferred to a test tube containing 9 ml of Tween 80® solution in 0.1% v/v. The amount of conidia or SPO was determined by counting in a Neubauer chamber. The conidial viability or GER was determined as described by Francisco et al. (2006).

Pathogenicity trials of *B. yamadae* formulation on wild poinsettia

Based on the results of the compatibility test, the adjuvants and their respective concentrations were selected to compose the *B. yamadae*-based formulation and to evaluate its efficiency on weed plants.

In the greenhouse, *E. heterophylla* seeds were sown into plastic trays containing an organic substrate. After 20 days, the seedlings were transferred to 400 mL volume plastic pots containing sieved soil. Three seedlings were transferred to each plastic pot, and these were considered repetitions within each treatment to obtain a total of 15 plants per treatment.The plants at the four- to six-leaf stages were sprayed with 50.0 mL ofwater or control; adjuvants only or mixture; fungus formulation or *B. yamadae* + adjuvants and fungus onlyor *B. yamadae* with no adjuvants. Immediately after spraying, the plants were covered with plastic bags for 24 h. The incidence of disease was evaluated 10 days after spraying by scoring the

Table 1. Chemical adjuvants evaluated for compatibility with *B. yamadae* to compose a fungal bioformulation.

Adjuvant	Concentrations (%)	Function
Silicon dioxide	0.01; 0.05; 0.1; 0.5; 1; 2; 3	Anti-wetting agent
Supragil WP®	0.01; 0.05; 0.1; 0.2; 0.3; 0.4; 0.5	Dispersing agent/ Wetting agent
Supragil MNS 85®	0.01; 0.05; 0.1; 0.5; 1	Dispersing agent
Geropon T 36®	0.01; 0.05; 0.1; 0.2; 0.3; 0.4; 0.5	Dispersing agent, Compatibility agent
Silicone oil	0.05; 0.075; 0.1; 0.15; 0.2	Dispersing agent, Spreading agent
Geropon SDS®	0.5; 1; 1.5; 2; 3	Wetting agent for dry formulations
Rhodapon LS 94 RPB®	0.5; 1; 1.5; 2; 3	Anionic surfactant

Table 2. Anti-wetting effect of silicon dioxide at several doses on the viability, mycelia growth, andsporulation of *B. yamadae* and the respective toxicological classifications.

Concentration	Germination (%)	Growth (mm)	Sporulation ($x10^6$ g substrate^{-1})	BI	Toxicological classification
Control	100	90 [a]	47.9 [a]		
0.01%	100	90 [a]	68.5 [a]	127	C
0.05%	100	90 [a]	39.1 [a]	97	C
0.10%	100	90 [a]	37.7 [a]	96	C
0.50%	100	90 [a]	55.5 [a]	115	C
1.00%	100	90 [a]	43.2 [a]	101	C
2.00%	100	85 [a]	59.0 [a]	115	C
3.00%	100	68 [a]	53.5 [a]	126	C
F test	-	2.17 ns	1.47ns	-	-
C.V. (%)	-	3.56	9.78	-	-

Original values and statistical analysis of sporulation and germination performed with log x and arc sin (x/100) data transformation, respectively. Means followed by the same letter in the column do not differ by the Tukey test ($p \geq 0.05$). ns: Not significant. BI: Biological index; C.V.: coefficient of variation; C: compatible.

number of leaves with symptoms and the total number of leaves on each plant according to De Nechet et al. (2006).

Statistical analysis

All data were submitted to variance analysis by the F-test, and the means were compared by Tukey's test with 5%probability, using AgroEstat software (Barbosaand Júnior-Maldonado, 2015).

RESULTS AND DISCUSSION

Anti-wetting silicon dioxide was previously identified by Machado et al. (2016) as a wettable powder for *B. yamadae* bioformulation due to its texture, which allows an increase in the preparation volume of conidia + anti-wetting and creates a homogeneous mixture. In the present study, the concentration used by these authors was extrapolated to verify the level of fungal tolerance to the product. All the evaluated parameters did not differ significantly from the control. Fungal sporulation varied between 37.7 and 68.5 x 10^6 conidia.g substrate^{-1}, and all the concentrations were considered compatible with the phytopathogen according to the BI model as shown in Table 2.

Different concentrations of the dispersant agents led to significant differences in all the parameters evaluated, except for *B.yamadae* germination and sporulationwith Geropon T 36®as presented in Table 3.When both Supragil WP® and Supragil MNS 85® were added to the culture medium, the biological parameters evaluated were inversely proportional to the increase in the product concentration in the medium, starting from 0.3 to 0.5% and fungal growth and development were completely inhibited (Table 3). These products were considered moderately toxic and toxic to the fungus, except Supragil WP®at 0.01% concentration.

For the compatibilizer agent Geropon T36®, even though a reduction in the diameter of the colonies was observed after ten days of incubation, the fungal sporulation was approximately 10^6 conidia.ml^{-1}for all evaluated concentrations. However, according to the BI,

Table 3. Effects of dispersants at several doses on the viability, mycelial growth, and sporulation of *B. yamadae*, and their toxicological classifications.

Product and concentration	Germination (%)	Growth (mm)	Sporulation ($x10^6$ con g substrate^{-1})	BI	Toxicological classification
Supragil WP®					
Control	99.5^a	90.0^a	2.89^a	-	-
0.01%	99.6^a	90.0^a	2.64^a	96	C
0.05%	97.7^a	38.0^b	1.55^{ab}	42	MT
0.1%	91.9^a	29.7^c	0.85^b	48	MT
0.2%	35.1^b	20.0^d	0.25^c	18	T
0.3%	0.0^c	0.0^e	0.0^d	0	T
0.4%	0.0^c	0.0^e	0.0^d	0	T
0.5%	0.0^c	0.0^e	0.0^d	0	T
F test	106.56*	38762.18**	88.38**	-	-
C.V. (%)	15.13	0.87	19.63	-	-
Supragil MNS 85®					
Control	99.6^a	90.0^a	41.3^a	-	-
0.01%	97.8^b	22.3^b	24.3^{ab}	47	MT
0.05%	77.2^c	18.3^{bc}	6.4^b	24	T
0.1%	44.1^d	15.8^c	8.8^{ab}	22	T
0.5%	0.0^e	0.0^d	0.0^c	0	T
1.0%	0.0^e	0.0^d	0.0^c	0	T
F test	1445.96*	1995.12**	23.10**	-	-
C.V.(%)	3.49	3.67	35.81	-	-
Geropon T36®					
Control	100	90.0^a	30.3^a	-	-
0.01%	100	90.0^a	22.3^a	89	C
0.05%	100	78.0^b	23.8^a	84	C
0.1%	100	70.4^c	19.3^a	74	C
0.2%	100	68.3^c	13.3^a	64	MT
0.3%	100	52.8^e	13.8^a	57	MT
0.4%	100	57.9^d	11.5^a	60	MT
0.5%	100	50.0^e	17.5^a	61	MT
F test	-	170.59**	0.68ns	-	-
C.V. (%)	-	0.83	25.09	-	-
Silicone oil					
Control	99.7^a	90.0^a	24.3^a	-	-
0.05%	98.9^b	90.0^a	26.0^a	103	C
0.075%	98.9^b	90.0^a	12.8^{ab}	80	C
0.1%	98.9^b	59.3^b	6.7^b	53	MT
0.15%	98.6^b	36.0^c	5.2^b	38	T
0.2%	98.3^b	35.0^c	1.1^c	30	T
F test	6.85**	1347.01**	25.17**	-	-
C.V. (%)	1.37	0.6	22.68	-	-

Original values and statistical analysis of sporulation and germination performed with log x and arc sin (x/100) data transformation, respectively. Means followed in the column by at least one of the same letter do not differ by the Tukey test ($p \geq 0.05$). ns: Not significant. BI: Biological index; C.V.: coefficient of variation; C: compatible; MT moderately toxic; T toxic.

only concentrations of 0.01, 0.05 and 0.1% were considered compatible with the fungus (Table 3). The compatibilizing function of this product in the formulation is essential, as it improves the homogeneity of the mixture and the uniformity of application (McMullan, 2000), ensuring that all plant leaves got into contact with the inoculum.

Silicone at concentrations of 0.05 and 0.075% was also

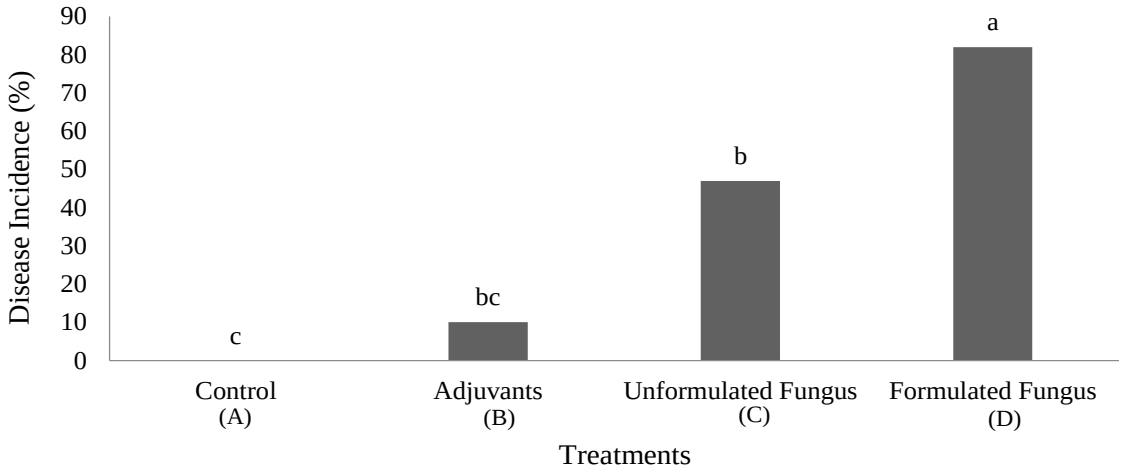

Figure 1. Effect of *B. yamadae* bioformulation on *E. heterophylla.* Disease incidence on *E. heterophylla* (%) after 10 days of spraying with solutions without infectious propagules of *B. yamadae* (A and B) and containing *B. yamadae* (C and D).

classified as compatible with the fungus and could be used in bioherbicide formulations (Table 3).The products Geropon SDS® and Rhodapon LS 94® at concentrations of 0.5 to 3.0% completely inhibited conidial germination in *B. yamadae* and, consequently, the subsequent stages of fungal development (data not shown). For the chemical control of weedy plants, the presence of adjuvants in the spraying tank is essential; even though adjuvants do not directly affect the efficacy of theherbicide, they improve the efficacy of pesticides by reducing or minimizing any negative effects at the time of application (McMullan, 2000). These effects are desirable in bioproduct development for pest control and justify the importance and benefits of adjuvants associated with biopathogens of interest. However, there are no guidelines for the selection of adjuvants to be used in association with biocontrol agents, which leads to the necessity of investigating and selecting compatible products for different phytopathogen-weed systems (Sanyal et al., 2008).

Several studies have shown the association effects of adjuvants and pathogens on the control of different target weeds (Gronwald et al., 2002; Borges-Neto et al., 1998; Borges-Neto and Pitelli, 2004). However, these studies did not evaluate the effects of these products on the bioagent, which can explain the failure of weed control in some cases. Only a few studies showed that the adjuvants had negative or toxic effects on the biopathogens, and this was the main motivation for the present study.

Similarly, Zhang et al. (2003) evaluated the effects of different surfactants and adjuvants on the germination and mycelial growth of *Colletotrichum* sp. and *Phoma* sp. in order to compose bioformulations with these fungi as phytopathogens. The results showed varied effects on the evaluated parameters, with increased or decreased effect depending on the situation.Based on the results of the present study, the wettable powder formulation with *B. yamadae* was composed of two distinct fractions: (1) a powder fraction and (2) a water fraction. The powder fraction was composed of the active ingredient, or conidia,at a final concentration of 10^7 conidia.ml^{-1} with anti-wetting silicon dioxide at 1% and inert kaolin, added only to increase the volume of the fraction. The water fraction was composed of 0.1% Geropon T36® the compatibilizer agent, 0.075% siliconeor dispersant, 0.1% Tween 80 or surfactant and 0.5% PVP K30® or spreading agent. The latter two products were identified as nontoxic to *B. yamadae*in previous studies (Machadoe et al., 2013, 2016).

In the greenhouse, the variance analysis was significant ($p>0.05$) in the test that evaluated the fungal pathogenicity on poinsettia. The incidence of disease was observed on 46.5 and 83.6% of the plants sprayed with a solution containing the bioagent alone and the formulated bioagent, respectively (Figure 1). Additionally, the mixture containing only the adjuvants did not cause a notable incidence of disease, but only 11.2% when sprayed on the plants, confirming that the adjuvantswere not toxic to this weed thus reaffirming the phytopathogenic action of the fungus (Figure 1).

The plants in the treatments containing the biological agent presented necrotic spots on leaves and stems within 48h after spraying. Moreover, during the evaluation period, intense defoliation thatresultedinto the death of the aerial partsof the plants was observed. Among the adjuvants that are being researched for use in fungal-based formulations, surfactants, when added to the spray solution, promote the suspension, dispersion, deposition, wetting, adhesion and retention of the conidia, thereby increasing the toxicity to the target (Costa et al., 2003).

Comparing the treatments in which the poinsettia

weeds were sprayed with solutions containing formulated and non-formulated microorganisms, the chemical adjuvants incorporated with the fungus were able to increase the contact and interaction of the fungus with the target plant, allowing significantly more effective control (79.0%). These products might have contributed in changing the morphology of the epicuticular wax or caused leaf tissue injuries, thereby facilitating *B. yamadae* entry and development.

B. yamadae produces a specific phytotoxin against wild poinsettia that causes negative effects during germination and affects susceptible leaves, promoting intense defoliation, but it does not affect non-host crops (Barbosa et al., 2002). It has been reported that fungal phytotoxins may also interact with plants other than the specific host (Hudson, 1986); however, this was not the case for *B. yamadae*in the present study as reported by Barbosa et al. (2002). This fungus did not affect soybean germination, and no disease symptoms were observed during soybean development (data not shown).

Conclusion

Bipolaris yamadae development was influenced by the tested adjuvants, including by varying the fungal response at different concentrations of these products. After the compatibility tests, a *B. yamadae* bioformulation was developed containing 1% silicon dioxide or anti-wetting, 0.1% Geropon T36® as compatibilizer agent, 0.075% silicone or dispersant, 0.1% Tween 80®or tensioactive agent and 0.5% PVP K30®or spreading agent. The test of the bioformulation against *E. heterophyllla* showed that weed control was improved when the plants received formulated *B. yamadae* compared to that under its direct, unformulated inoculation. Therefore, this study presents the development of a new phytopathogenic fungus-based formulation with great efficiency as a bioherbicidal agent for the control of poinsettia weeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Barbosa AM, Souza CG, Dekker RF, Fonseca RC, Ferreira DT (2002). Phytotoxin produced by *Bipolaris euphorbiae* in-vitro is effective against the weed *Euphorbia* heterophylla. Brazilian Archives of Biology and Technology 45(2):233-240.

Barbosa JC, Júnior-Maldonado W (2015). Sistemas para análises estatísticas de ensaios agronômicos (AgroEstat). Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal.

Borges-Neto CR, Mello SCM, Ribeiro ZMA, Fontes EG (1998). Efeito de adjuvantes no crescimento e infectividade do fungo *Cercospora caricis*, agente de biocontrole da tiririca. Fitopatologia Brasileira 23(4):502-502.

Borges-Neto CR, Pitelli RA (2004). Adjuvantes e herbicidas e a infectividade de *Fusarium graminearum*, agente potencial de biocontrole de *Egeria densa* e *Egeria najas.*Planta Daninha 22(1):77-83.

Caldwell CJ, Hynes RK, Boyetchko SM, Korber DR (2012).Colonization and bioherbicidal activity on green foxtail by *Pseudomonas fluorescens* BRG100 in a pesta formulation. Canadian Journal of Microbiology 58(1):1-9.

Costa EAD, Almeida JEM, Loureiro ES, Sano AH (2003). Compatibilidade de adjuvantes no desenvolvimento "in vitro" dos fungos entomopatogênicos *Metarhizium anisopliae* (Metsch) Sorokin e *Beauveria bassiana* (Bals) Vuillemin. Revista STAB 22:38-40.

De Nechet KL, Barreto RW, Mizubuti ES (2006). *Bipolaris euphorbiae* as a biological control agent for wild poinsettia (*Euphorbia heterophylla*): host-specificity and variability in pathogen and host populations. BioControl 51(2):259-275.

Falk RH, Guggenheim R, Schulke G (1994). Surfactant-induced phytotoxicity Weed Technology 8(3):519-525.

Francisco EA, Mochi DA, Correia ADCB, Monteiro AC (2006). Influence of culture media in viability test of conidia of entomopathogenic fungi. Ciência Rural 36(4):1309-1312.

Greaves MP, MacQueen MD (1990). The use of mycoherbicides in the field. Aspects of Applied Biology 24:163-168.

Green JM (2014). Current state of herbicides in herbicide-resistant crops. Pest Management Science 70:1351-1357.

Gronwald JW, Plaisance KL, Ide DA, Wyse DL (2002). Assessment of *Pseudomonas syringae*pv*tagetis* as a biocontrol agent for Canada thistle. Weed Science 50(3):397-404.

Hudson HJ (1986). Fungi as parasitic symbionts of plants, in: R Arnold, Fungal Biology. London, pp. 262-276.

Keswani C, Bisen K, Singh V, Sarma BK, Singh HB (2016). Formulation technology of biocontrol agents: present status and future prospects. In: Bioformulations: For sustainable agriculture, Springer, New Delhi, pp. 35-52.

Loureiro EDS, Batista Filho A, Leite LG, de Almeida JEM (2003). Viabilidade de conídios e blastósporos de *Sporothrix insectorum* (Hoog & Evans) armazenados em diferentes temperaturas. Arquivos do Instituto Biológico 70(1):89-91.

Machado ACR, Mochi DA, Monteiro AC (2013). Crop optimization and pre-steps standardization to get a *Bipolaris euphorbiae*-based bioherbicide. Pesquisa Agropecuária Tropical 43(4):392-399.

Machado ACR, Monteiro AC, Mochi DA (2016).Selection of anti-wetting agents and photo protectants compatible with a soybean weed control bioagent. African Journal of Microbiology Research 10(43):1829-1836.

McMullan P (2000).Utility Adjuvants.Weed Technology 14(4):792-797.

Marin-Felix Y, Senwanna C, Cheewangkoon R, Crous PW (2017).New species and records of *Bipolaris* and *Curvularia* from Thailand. Mycosphere 8(9):1556-1574.

Prasad R (1994). Influence of several pesticides and adjuvants on *Chondrostere umpurpureum*- a bioherbicide agent for control of forest weeds. Weed Technology 8:445-449.

Penariol MC, Monteiro AC, Pitelli RA, Pereira GT (2008). Produção de *Bipolaris euphorbiae* em meios de cultura sólidos e líquidos obtidos de grãos e resíduos agroindustriais. Bragantia 67(4):805-814.

Pontecorvo G, Roper JA, Chemmons LM, MacDonald KD, Bufton AWJ (1953). The genetics of *Aspergillus nidulans*. Advances in Genetics 5:141-238.

Rossi-Zalaf LS, Alves SB, Lopes RB, Silveira Neto S, Tanzini MR (2008). Interação de microrganismos com outros agentes de controle de pragas e doenças. In: *C*ontrole microbiano de pragas na América Latina: avanços e desafios, FEALQ, pp. 279-302.

SanyalD, Bhowmik PC, Abbas HK (2008). Effect of Surfactants on Bioherbicidal Activity of *Alternariahelianthi* on Multiple-Seeded Cocklebur. The Plant Pathology Journal 7(1):104-108.
Womack JG, Burge MN (1993). Mycoherbicide formulation and the potential for bracken control. Pesticide Science 37:337-341.
Wraight SP, Jacksonz MA, De Kock SL (2001). Formulation of Fungal Biocontrol Agents Fungi as biocontrol agents: Progress problems and potential 253.
WyssGS, Charudattan R, Rosskopf EN, Littell RC (2004).Effects of selected pesticides and adjuvants on germination and vegetative growth of *Phomopsis amaranthicola*, a biocontrol agent for *Amaranthus* spp. Weed Research 44(6):469-482.
ZhangW, Wolf TM, Bailey KL, Mortensen K, Boyetchko SM (2003). Screening of adjuvants for bioherbicide formulations with *Colletotrichum* spp and *Phoma* spp. Biological Control 26(2):95-108.
Zhu H, Ma Y, Guo Q, Xu B (2020). Biological weed control using *Trichoderma polysporum* strain HZ-31. Crop Protection 134:105161.