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Selection of anti-wetting agents and photoprotectants compatible with a soybean weed control bioagent

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Adjuvants incorporation at formulations can positively influence the performance of bioagent control, contributing to preservation of the inoculum until being used. Adjuvants should not be toxic to pathogen and tests that evaluate the sensitivity of biologic products are essential. The aim of this study was to analyze the compatibility of the anti-wetting agents microcrystalline cellulose, anhydrous sodium carbonate, magnesium oxide, talc, calcium carbonate, silicon dioxide, and photoprotectants NeoHeliopan® AV, NeoHeliopan® E1000, Eusolex® 6007, NeoHeliopan® Hydro, Tinosorb® M, Eusolex® 232 and a Complex filters UVA/UVB with the fungus *Bipolaris euphorbiae*, in order to select products to formulate a fungus-based bioherbicide. All products were used in concentrations of 0.01, 0.05, 0.1, 0.5 and 1%. After evaluating vegetative growth, sporulation, and germination, the products were toxicologically classified by calculating the biological index. The anti-wetting agents calcium carbonate, talc, microcrystalline cellulose, and silicium dioxide did not interfere in the development of the fungus, and were deemed compatible. The photoprotectant Tinosorb® M was classified as compatible with the fungus in all concentrations used, a similar outcome to Eusolex® 6007, except at 1.0% concentration. Most of the other photoprotectants were compatible in concentrations ranging between 0.01 and 0.1%, except for Complex filters UVA/UVB at 0.05% and NeoHeliopan® E1000 at 0.1%. NeoHeliopan® Hydro was rated moderately toxic to the fungus in all concentrations used. Conidial germination was less affected than growth and sporulation.

Key words: *Bipolaris euphorbiae*, biological control, bioproduct, formulation, adjuvants.

INTRODUCTION

Bipolaris euphorbiae is a specific pathogen of *Euphorbia heterophylla* L. (milkweed), one of the most significant weeds affecting soybeans. In order to be used as a biological control agent, it is necessary to develop a fungus-based bioproduct.

Development of bio-herbicides requires finding appropriate technologies for mass production, formulation, and preservation of the inoculum until the use phase (Tessmann, 2011). One of the aspects that limits the advances in this type of control is obtaining appropriate

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Table 1. Chemical adjuvants assessed in this study with respect to compatibility with *Bipolaris euphorbiae*.

Function	Trade name	Chemical name or composition
Anti-wetting agent	Microcrystalline cellulose	-
	-	Anhydrous sodium carbonate
	-	Magnesium oxide
	Talc	Hydrated magnesium silicate
	-	Calcium carbonate
	-	Silicium dioxide (Pyrogenic silica)
Photoprotectant	NeoHeliopan® AV	Octyl methoxycinnamate
	NeoHeliopan® E1000	Isoamyl-methoxycinnamate
	Eusolex® 6007	Ethylhexyl dimethyl PABA
	NeoHeliopan® Hydro (Symrise)	Phenylbenzimidazole sulfonic acid
	Tinosorb® M	Methylene-bis-benzotriazolyl tetramethylbutylphenol
	Eusolex® 232 (Merck)	Phenylbenzimidazole sulfonic acid
	Complex filters UVA/UVB	Benzophenone 4 + phenylbenzimidazole sulfonic acid

formulations. Formulation is the way in which the active ingredient is presented in an effective physical form. It begins with the production of the pathogen, and continues through the addition of products (adjuvants) that aim to stabilize the biological agent during storage, facilitate product handling and application, protect the bioagent from environmental factors, and increase pathogen activity, boosting its reproduction, contact and interaction with the target host (Morentini and Melo, 2007; Almeida et al., 2008).

Mass production of *B. euphorbiae* and the pre-stages in obtaining a bio-herbicide have already been investigated and established (Penariol et al., 2008a; Machado et al., 2013; Moraes et al., 2014). However, it is important to find adjuvants compatible to the pathogen which promotes improvements in one or more characteristics essential to establishing the antagonistic relationship (Fravel et al., 1998) that favor the development of a formulation. Among adjuvants that are usable in formulating myco-herbicides, anti-wetting agents that keep the level of available water low, that prevent fungus conidia from germination when stored, stand out. Therefore, in order for a bioproducts to be commercially competitive, it is crucial to extend their useful life, to increase the period that the pathogen propagules can be stored, while remaining viable and infectious (Elzein et al., 2008).

Solar radiation is one of the most significant environmental problems affecting the efficiency of fungi as biological control agents (Braga et al., 2006), since sensitivity to radiation limits the use of such biological control agents in field conditions (Francisco et al., 2008). When conidia are exposed to ultraviolet radiation (UV), the cytoplasmic membrane and cellular organelles are subject to degradation, along with several other direct effects on the DNA, resulting in delayed germination or complete inactivation (Rangel et al., 2006; Chelico and Khachatourians, 2008). Photoprotectants are substances that are able to absorb and/or disperse UV rays, according to their structure, protecting against the adverse effects of this radiation. Therefore, it is important to find photoprotectants that are compatible with *B. euphorbiae* so they can be added to the formulation.

Adding adjuvants to formulations can influence conidia performance positively. However, the adjuvants may be toxic to the biological control agent, so it is essential to evaluate toxicity to the specific microorganism (Wyss et al., 2004).

The aim of this study was to analyze the compatibility of anti-wetting agents and photoprotectants with fungus *B. euphorbiae,* aiming to select products to formulate a fungus-based bio-herbicide.

MATERIALS AND METHODS

For use in the tests, the FCAV#569 isolate of *B. euphorbiae* was grown in Petri dishes containing the Pontecorvo minimal medium, modified by supplementation with peptone (2 g L^{-1}) and with the substitution of glucose by starch (10 g L^{-1}) (Penariol et al., 2008b). The fungus was kept at 25 ± 0.5°C for 10 days, with a 12-h photoperiod.

The adjuvants assessed and their functions are shown in Table 1. All products were used in arbitrarily defined concentrations of 0.01, 0.05, 0.1, 0.5 and 1%. Vials containing the liquid Pontecorvo minimal medium, received predetermined quantities of the adjuvants, and next, the media were transferred into Petri dishes. Since they are liposoluble, the photoprotectants NeoHeliopan® AV, NeoHeliopan® E1000 and Eusolex® 6007 were mixed in sterile solution of arabic gum (0.7% w v^{-1}) for emulsification before being added to the culture medium. To increase solubilization and stabilize pH, the photoprotectants NeoHeliopan® Hydro and Eusolex® 232 were added along with Triethanolamine (1:1 v v^{-1}), which was pre-tested for compatibility with the fungus. The control treatment was composed of the minimal medium without the addition of any product. After the culture medium solidified, the inoculation was conducted, by transferring to the center of the Petri

dish a 5 mm diameter disc of fungus culture obtained from colonies with 10 days of growth. Then, *B. euphorbiae* colonies were maintained in the incubation conditions described earlier.

The vegetative growth, sporulation, and conidia germination were assessed. Vegetative growth was analyzed by measuring (in mm) two perpendicular diameters on the 10th day of incubation. After this period, the conidia formed on the surface of the colony were removed by scraping and transferred to a test tube containing 9 ml of Tween 80® solution (0.1% v v^{-1}). From this suspension, conidia number was determined by counting in a Neubauer chamber. Germination was assessed by micro-cultivation on slides and direct microscopic examination, according to the methodology described by Francisco et al. (2006). Three areas were marked microscope slides, and the surface was covered with 4 ml of minimal medium containing the products in the respective concentrations. In the region of the culture medium, a drop of a fungal suspension (1 × 10^6 con. ml^{-1}) was inoculated, and incubated at 25 ± 0.5°C for 7 h. One hundred and fifty conidia were observed, germinated and non-germinated, in each area, thus establishing a percentage ratio of viable conidia.

Tests were conducted using a completely randomized design (CRD) composed of 4 repetitions. Data were subjected to variance analysis using the F test, and the means were compared using the Tukey test at 5% probability. The AgroEstat program was used for the statistical tests

To determine the toxicity of the adjuvants for the fungus, the biological index (BI) model was used, which is described by Rossi-Zalaf et al. (2008) and calculated by the formula:

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

where VG = percentage of vegetative growth in the colony after 10 days of incubation as compared to the control, SP = percentage of sporulation after 10 days of incubation compared to the control, GER = percentage of germination of the conidia after 7 h of incubation, in relation to the control.

Using the BI values, adjuvants toxicity was classified using the scale described by Rossi-Zalaf et al. (2008), where BI from 0 to 41 = toxic; BI from 42 to 66 = moderately toxic; and BI > 66 = compatible.

RESULTS AND DISCUSSION

Different concentrations of the anti-wetting agents calcium carbonate, talc, microcrystalline cellulose, and silicon dioxide did not affect the development of *B. euphorbiae,* especially with regard to vegetative growth and germination, being considered compatible with the fungus (Tables 2 and 3). Therefore, these products can be used in formulating fungus-based bio-herbicides at any of the tested concentrations.

In the treatments containing 0.5 and 1% of anhydrous sodium carbonate and magnesium oxide, all of the evaluated parameters were affected, but the germination obtained in the presence of magnesium oxide (>93%) can be considered satisfactory (Tables 2 and 3, respectively), suggesting that initially the fungus used endogenous sources of macronutrients that allowed the germination of conidia. Then they used exogenous sources contained in the culture medium with the anti-wetting and causing the deleterious effect. In these concentrations, both products were considered toxic to the fungus. Anhydrous sodium carbonate was classified as compatible with the fungus only in concentrations of 0.01 and 0.05% (Table 2), and the magnesium oxide was deemed compatible in concentrations of 0.01 to 0.1%, as it had little or no significant effect on the performance of the bioagent (Table 3).

The development of formulations based on biological agents is very similar to processes in the pharmaceutical, cosmetics, and food industries, in terms of searching for ingredients that are safe, inexpensive, and non-toxic (Gaugler, 1997). The products tested in this study are commonly used in the food industry and are classified under Brazilian law as safe for human consumption.

Results of studies addressing fungi pathogenic to weeds associated with anti-wetting agents for use in formulating bio-herbicides have not been found in the literature, which makes it impossible to compare the results obtained in this study with those of other authors. Therefore, these results are important because they represent the first data obtained for these topics.

Among the parameters used to evaluate the compatibility of photoprotectants with *B. euphorbiae*, sporulation of the fungus was more affected than the control (Tables 4 and 5). Growth and germination were less affected by the action of most of the products tested, although there were significant differences compared to the control in some treatments.

NeoHeliopan® AV was classified as compatible with the fungus when used at 0.01, 0.05 and 0.1%, but completely inhibited growth, sporulation, and germination at concentrations of 0.5 and 1.0%, and was classified as toxic (Table 4). The chemical derivative contained in this commercial product is one of the most commonly used to protect against electromagnetic spectrum UVB radiation, and Brazilian law permits its use at concentrations varying from 2 to 7.5% for pharmaceutical formulations. The concentrations used in the present study, were considerably lower than these levels, but the product was only compatible with the fungus up to 0.1% concentration (Table 4).

The photoprotectant NeoHeliopan® E1000 affected the performance of the fungus in concentrations of 0.1%to 1.0%, and was classified as moderately toxic, but proved to be compatible at concentrations of 0.01 and 0.05% (Table 4). Eusolex® 6007 showed no deleterious effect on growth and germination, affecting only sporulation. Consequently, it was classified as compatible with the fungus at all concentrations, except 1.0% (Table 4). NeoHeliopan® Hydro did not affect germination of *B. euphorbiae*, had little effect on growth, and most impacted sporulation. Consequently, it was classified as moderately toxic to the fungus at all of the assessed concentrations (Table 5). In the presence of Tinosorb® M, growth, sporulation, and germination of *B. euphorbiae* were unaffected, with no difference in relation to the control. Based on these data, the calculation of the

Table 2. Toxicity of the anti-wetting agents calcium carbonate, talc, and anhydrous sodium carbonate to *B. euphorbiae* grown in culture medium containing different concentrations of products.

Anti-wetting agents and concentrations	Growth (mm)	Sporulation (n° of con. × 10^6)	Germination (%)	BI	Toxicological classification
Calcium carbonate					
Control	90.0	4.8^{A}	95.6^{A}	-	-
0.01%	90.0	3.6^{A}	93.5^{AB}	90	C
0.05%	90.0	8.0^{A}	94.9^{AB}	129	C
0.1%	90.0	4.8^{A}	94.4^{AB}	100	C
0.5%	90.0	3.6^{A}	95.0^{A}	79	C
1.0%	90.0	6.3^{A}	92.2^{B}	113	C
F test	-	2.44^{ns}	4.74*	-	-
C.V. (%)	-	9.26	1.56	-	-
Talc					
Control	90.0	10.5^{A}	99.9^{A}	-	-
0.01%	90.0	7.0^{AB}	99.6^{A}	86	C
0.05%	90.0	8.0^{AB}	99.6^{A}	90	C
0.1%	90.0	2.5^{C}	99.9^{A}	67	C
0.5%	90.0	4.5^{BC}	99.8^{A}	75	C
1.0%	90.0	4.0^{BC}	99.9^{A}	77	C
F test	-	5.67**	0.47^{ns}	-	-
C.V. (%)	-	10.61	2.45	-	-
Anhydrous sodium carbonate					
Control	90.0^{A}	9.0^{A}	99.8^{A}	-	-
0.01%	90.0^{A}	4.3^{B}	99.0^{A}	67	C
0.05%	90.0^{A}	4.5^{B}	98.6^{A}	78	C
0.1%	90.0^{A}	1.1^{C}	99.3^{A}	62	MT
0.5%	20.0^{B}	0.2^{C}	62.4^{B}	18	T
1.0%	0.0^{C}	0.0^{C}	7.7^{C}	1	T
F test	45844.64**	20.52**	791.43**	-	-
C.V. (%)	0.62	9.16	2.60	-	-

Original values and statistical analysis of sporulation and germination performed with data transformed into log x and arc sin (x/100), respectively. Means followed in the column by at least one common letter do not differ by the Tukey test ($p \geq 0.05$). ^{ns}Not significant; ** ^{and} *Significant at 1 and 5% probability, respectively. BI: Biological index. CV: coefficient of variation. C: compatible; MT: moderately toxic; T: toxic.

biological index ranged from 81 to 95, classifying this product as compatible with the fungus in all concentrations tested (Table 5).

This photoprotectant is considered to be a cutting-edge product, and is widely used around the world. It is an organic solar filter made of microfine particles whose protective action against UV radiation consists of absorption, reflection and dispersion of solar radiation (Lim et al., 2005). Furthermore, it is photostable, an important feature in developing formulations containing solar filters, since they can interact with other compounds in the formulation that may be degraded as a result of UV exposure (Wissing and Muller, 2001). Eusolex® 232 and the Complex filters UVA/UVB had little or no effect on growth and germination, and were classified as compatible with the fungus in concentrations of 0.01 to 0.1%, except for the Complex filters UVA/UVB at 0.05%. In this concentration, the product was classified as moderately toxic, with a BI of 66, a value corresponding to the maximum limit for this classification, according to Rossi-Zalaf et al. (2008), and near the lower limit for classification as compatible. In other concentrations, both products were considered moderately toxic to the fungus, and sporulation was the most affected parameter (Table 5). Studies involving the compatibility of *B. euphorbiae* with photoprotectants were not found in the literature. Various concentrations of the photoprotectants Oxybenzone®, NeoHeliopan® AV and NeoHeliopan® E1000 had no deleterious effects on the germination of *Beauveria bassiana* conidia, and were considered

Table 3. Toxicity of the anti-wetting agents magnesium oxide, microcrystalline cellulose and silicon dioxide to *B. euphorbiae* grown in culture medium containing different concentrations of products.

Anti-wetting agents and concentrations	Growth (mm)	Sporulation (nº of con. × 10^6)	Germination (%)	BI	Toxicological classification
Magnesium oxide					
Control	90.0^{A}	5.8^{A}	99.9^{A}	-	-
0.01%	90.0^{A}	6.0^{A}	99.5^{A}	102	C
0.05%	90.0^{A}	7.3^{A}	99.7^{A}	111	C
0.1%	90.0^{A}	5.5^{A}	93.6^{B}	97	C
0.5%	23.3^{B}	0.2^{B}	93.2^{B}	23	T
1.0%	6.8^{C}	0.0^{B}	93.7^{B}	13	T
F test	56.64**	15.33**	74.18**	-	-
C.V. (%)	11.87	9.62	1.63	-	-
Microcrystalline cellulose					
Control	90.0	1.7^{A}	99.6^{A}	-	-
0.01%	90.0	1.4^{AB}	99.5^{A}	93	C
0.05%	90.0	1.6^{A}	99.7^{A}	103	C
0.1%	90.0	1.3^{AB}	99.6^{A}	90	C
0.5%	90.0	1.1^{AB}	99.5^{A}	84	C
1.0%	90.0	0.9^{B}	99.1^{A}	88	C
F test	-	4.12*	0.23^{ns}	-	-
C.V. (%)	-	11.46	2.66	-	-
Silicon dioxide					
Control	90.0	1.65^{A}	98.2^{A}	-	-
0.01%	90.0	1.68^{A}	97.2^{A}	100	C
0.05%	90.0	0.93^{A}	97.0^{A}	81	C
0.1%	90.0	0.90^{A}	97.6^{A}	80	C
0.5%	90.0	0.85^{A}	97.9^{A}	69	C
1.0%	90.0	0.78^{A}	97.4^{A}	77	C
F test	-	3.39*	1.12^{ns}	-	-
C.V. (%)	-	3.54	1.46	-	-

Original values and statistical analysis of sporulation and germination performed with data transformed into log x and arc sin (x/100), respectively. Means followed in the column by at least one common letter do not differ by the Tukey test ($p \geq 0.05$). ^{ns}Not significant; $^{**\text{ and }}$*Significant at 1 and 5% probability, respectively. BI: Biological index; CV: coefficient of variation; C: compatible; MT: moderately toxic; T: toxic.

compatible with the fungus (Santos et al., 2011). However, this assessment did not consider the BI model, which includes data on vegetative growth and sporulation.

The adverse effects of solar radiation on germination of conidia have been reported by several authors (Rangel et al., 2004; Rangel et al., 2005; Braga et al., 2006). These effects reduce the activity of the fungus on the host (Rangel et al., 2006; Chelico and Khachatourians, 2008). Conidia of *B. euphorbiae* are tolerant to solar and ultraviolet radiation, and remain viable (germination > 92%) after 8 h of exposure to radiation emitted by a solar simulator or 90 min of exposure to germicidal UV radiation, without the use of any formulation (Moraes et al., 2011).

Among the protective factors to UV radiation intrinsic to conidia of fungi are the pigments located in the cell wall, which act to block the entrance of radiation (Rangel et al., 2005; Braga et al., 2006). Hyphae and conidia of fungi in the genus *Bipolaris* feature dark coloration due to the presence of melanin in the cell wall (Weikert-Oliveira et al., 2002). In microorganisms, the primary function of this pigment is to reduce the harmful effects of UV radiation on the cells. The correlation between melanin concentration and UV tolerance is being discussed. Melanin is also associated with protection against high temperatures and chemical stresses such as the presence of heavy metals and oxidizing agents (Allam and Abd El-Zaher, 2012). Although the results achieved by Moraes et al. (2011) have shown that *B. euphorbiae* is quite tolerant

Table 4. Toxicity of the photoprotectants NeoHeliopan® AV, NeoHeliopan® E1000 and Eusolex® 6007 to *Bipolaris euphorbiae* grown in culture medium containing different concentrations of products.

Photoprotectants and concentrations	Growth (mm)	Sporulation (nº of con. × 10^6)	Germination (%)	BI	Toxicological classification
NeoHeliopan®AV					
Control	90.0^{A}	1.6^{A}	88.3^{A}	-	-
0.01%	90.0^{A}	0.9^{AB}	80.0^{B}	80	C
0.05%	90.0^{A}	1.8^{A}	88.1^{A}	107	C
0.1%	60.8^{B}	1.7^{A}	84.3^{AB}	91	C
0.5%	0.0^{C}	0.0^{B}	0.0^{C}	0	T
1.0%	0.0^{C}	0.0^{B}	0.0^{C}	0	T
F test	164.14**	6.75**	1129.97**	-	-
C.V. (%)	12.51	6.06	3.02	-	-
NeoHeliopan® E1000					
Control	90.0^{A}	1.7^{A}	95.6^{A}	-	-
0.01%	90.0^{A}	1.4^{AB}	94.4^{AB}	92	C
0.05%	90.0^{A}	1.2^{ABC}	90.5^{B}	87	C
0.1%	63.3^{C}	0.6^{BC}D	81.9^{C}	56	MT
0.5%	66.8^{B}	0.3^{C}D	79.5^{C}	50	MT
1.0%	65.8^{B}	0.2D	78.1^{C}	48	MT
F test	559.03**	8.32**	39.26**	-	-
C.V. (%)	0.75	3.55	2.77	-	-
Eusolex® 6007					
Control	90.0	1.6^{A}	97.9^{A}	-	-
0.01%	90.0	1.0^{AB}	98.7^{A}	95	C
0.05%	90.0	0.7^{BC}	98.3^{A}	75	C
0.1%	90.0	0.6^{BC}	97.7^{A}	73	C
0.5%	90.0	0.4^{BC}	96.8^{A}	67	C
1.0%	90.0	0.2^{C}	96.8^{A}	62	MT
F test	-	8.50**	2.32^{ns}	-	-
C.V. (%)	-	2.93	1.99	-	-

Original values and statistical analysis of sporulation and germination performed with data transformed into log x and arc sin (x/100), respectively. Means followed in the column by at least one common letter do not differ by the Tukey test ($p \geq 0.05$). ^{ns}Not significant; **Significant at 1% probability. BI: Biological index. CV: coefficient of variation; C: compatible; MT: moderately toxic; T: toxic.

of solar radiation, it is useful to combine this phytopathogen with photoprotectants in order to increase its efficiency in field conditions, since sun exposure may surpass 8 h. A wide range of photoprotectants with potential for use in formulating bioproducts is available on the market, but studies investigating the compatibility of these photoprotectants with fungal control agents are scarce, especially weed pathogens.

Conclusions

B. euphorbiae is affected by the effect of anti-wetting agents and photoprotectants which can be used as adjuvants in formulating a fungus-based bioproduct. The development of the fungus is influenced by the concentration of the products used. Conidia germination is less affected by the products than vegetative growth and sporulation. In concentrations that are not toxic or moderately toxic to the fungus the anti-wetting agents and photoprotectants tested can be used to formulate a fungus-based bio-herbicide

Conflict of interests

The authors have not declared any conflict of interests.

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Table 5. Toxicity of the photoprotectants NeoHeliopan® Hydro, Tinosorb® M, Eusolex® 232 and Complex filters UVA/UVB to *Bipolaris euphorbiae* grown in culture medium containing different concentrations of products.

Photoprotectants and concentrations	Growth (mm)	Sporulation (nº of con. × 10^6)	Germination (%)	BI	Toxicological classification
NeoHeliopan® Hydro					
Control	99.0^{A}	7.8^{A}	87.7^{A}	-	-
0.01%	85.5^{AB}	4.1^{BC}	86.2^{A}	64	MT
0.05%	84.6^{BC}	3.5^{BC}	81.8^{A}	62	MT
0.1%	77.3^{D}	6.1^{B}	81.4^{A}	64	MT
0.5%	79.8^{CD}	1.3^{D}	81.8^{A}	52	MT
1.0%	78.3^{D}	2.1^{CD}	80.4^{A}	55	MT
F test	164.14**	22.54**	1.21^{ns}	-	-
C.V. (%)	12.51	9.22	6.15	-	-
Tinosorb® M					
Control	90.0	3.1^{A}	98.2^{A}	-	-
0.01%	90.0	1.7^{A}	98.8^{A}	81	C
0.05%	90.0	1.9^{A}	98.4^{A}	83	C
0.1%	90.0	2,7^{A}	98.7^{A}	95	C
0.5%	90.0	2.3^{A}	98.8^{A}	89	C
1.0%	90.0	1.8^{A}	98.7^{A}	82	C
F test	-	0.66^{ns}	0.30^{ns}	-	-
C.V. (%)	-	8.79	2.28	-	-
Eusolex® 232					
Control	90.0^{A}	1.8^{A}	97.6^{AB}	-	_
0.01%	90.0^{A}	0.8^{BC}	98.3^{A}	75	C
0.05%	90.0^{A}	0.7^{BC}	97.4^{AB}	75	C
0.1%	90.0^{A}	1.1^{AB}	97.4^{AB}	83	C
0.5%	90.0^{A}	0.4^{C}	96.6^{B}	55	MT
1.0%	65.5^{B}	0.3^{C}	97.0^{AB}	50	MT
F test	5461.68**	12.36**	2.32^{ns}	-	-
C.V. (%)	0.65	2.53	1.99	-	-
Complex filters UVA/UVB					
Control	90.0^{A}	3.2^{A}	98.4^{A}	-	-
0.01%	90.0^{A}	0.9^{B}	98.8^{A}	76	C
0.05%	90.0^{A}	0.7^{B}	98.9^{A}	66	MT
0.1%	90.0^{A}	0.8^{B}	98.6^{A}	68	C
0.5%	90.0^{A}	0.4^{B}	98.6^{A}	62	MT
1.0%	81.0^{B}	0.5^{B}	98.7^{A}	59	MT
F test	152.78**	5.82**	0.08^{ns}	-	-
C.V. (%)	0.34	6.42	2.13	-	-

Original values and statistical analysis of sporulation and germination performed with data transformed into log x and arc sin (x/100), respectively. Means followed in the column by at least one common letter do not differ by the Tukey test ($p \geq 0.05$). ^{ns}Not significant; **Significant at 1% probability. BI: Biological index; CV: coefficient of variation; C: compatible; MT: moderately toxic; T: toxic.

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