




# Association of Adjuvants with Culture Filtrate from *Fusarium fujikuroi* for Increasing the Control of *Conyza sp.*

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**Abstract:** Metabolites from *Fusarium fujikuroi* were used as a bioherbicide product for the control of *Conyza sp.*, a weed that causes considerable losses in the cultivation of soybean worldwide. Formulations containing distilled water or culture filtrate and different adjuvants (palm, soybean or mineral oil, Span® 80 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 3% (w/v) of mineral oil, 5.5% (w/v) of surfactant (Span® 80 and Tween® 80) and a hydrophilic-lipophilic balance (HLB) of 15, which resulted in a higher herbicidal activity (100%) (complete death of plants) and lower phytotoxicity (0%). The suitable combination of adjuvants in association with culture filtrate from *F. fujikuroi* increased up to 2.5 times the efficiency of bioherbicide for the post-emergence control of *Conyza sp.*

**Keywords:** bioherbicide; biotechnology process; submerged fermentation; formulation; weed control.

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## 1. Introduction

Weed control in agricultural areas has been one of the major challenges facing agriculture around the world for many decades. In Brazil, the *Conyza sp.*, popularly known as “buva”, has gained prominence in the soybean crop, mainly due to the presence of plants with highly competitive and dispersive potential, which could cause irreversible damage to the crop [1]. The control of *Conyza sp.* is usually accomplished through the use of chemical herbicides such as glyphosate. However, over the years, some species, including *Conyza sp.*, become resistant to the mechanism of action of certain chemicals [2]. 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 [3-8].

Taking into account this scenario, the prospection and discovery of new molecules appear as an important tool for the control of resistant weeds [9]. Some studies have shown promising results in the weed control using fermented broth containing the secondary

metabolites produced by fungi via submerged fermentation [10-14]. Secondary metabolites can damage weeds by penetrating the plant followed by the destruction of the cell wall and induction of necrotic lesions [15,16].

On the other hand, many promising biomolecules are early discarded during the stages of bioherbicide 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 [17]. Therefore, some strategies to concentrate these molecules are essential to obtain an efficient product. 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 [18,19,20]. This is highlighted in a study reported by Bastos et al. [21], in which the authors increased three times the herbicidal activity of culture filtrate from *Diaphorte sp.* obtained by solid state fermentation using 5.5 wt% of palm oil, 5.5 wt% of adjuvants and HLB 15.0.

The genus *Fusarium* has been widely used in the production of metabolites for weed control [22]. The herbicidal activity of different species (*Fusarium avenaceum*, *Fusarium acuminatum*, *Fusarium redolens*, *Fusarium culmorum*/*Fusarium cerealis* and *Fusarium solani*) was evaluated in different grass species, demonstrating promising results using *Fusarium avenaceum* and *Fusarium acuminatum* [23]. Recently, our research group isolated and identified the fungus *Fusarium fujikuroi* obtained from the Brazilian Pampa biome with herbicidal activity towards target plants, such as *Cucumis sativus* and *Sorghum bicolor* [24]. However, the potential of this strain for controlling resistant plants as *Conyza sp.* has not been investigated up to now. 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 fujikuroi*, isolated from the Pampa biome, for the control of *Conyza sp.*, a resistant weed in soybean crop.

## 2. Materials and Methods

### 2.1. Materials.

Corn steep liquor (CSL) was purchased from Ingredion (Mogi Guaçu, SP, Brazil). Palm oil (*Elaeis guineensis*) was provided by Agropalma (Tailândia, PA, Brazil). Soybean oil and sucrose (crystal) were purchased in a local market (Santa Maria, Brazil) and mineral oil Assist® was purchased from BASF S.A. (São Paulo, SP, Brazil). Silwet L-77 (FMC) and commercial substrate (Mecplant®) were purchased in a local agricultural supply store (Santa Maria, Brazil). Potato dextrose agar (PDA), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, FeSO<sub>4</sub>.7H<sub>2</sub>O, MnSO<sub>4</sub>.H<sub>2</sub>O, MgSO<sub>4</sub>, Tween® 80 and Span® 80 were purchased from Sigma-Aldrich (São Paulo, SP, Brazil).

### 2.2. Fermentation.

*Fusarium fujikuroi* was previously isolated by Daniel et al [24]. The culture was maintained in a petri dish with PDA medium between 4 °C and 6 °C and subcultured every 15 days. For pre-inoculum cell production was done incubating the culture on PDA in a Petri dish for 8 days at 28 °C. 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 (Innova 44R, New Brunswick, Canada) 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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.5), FeSO<sub>4</sub>.7H<sub>2</sub>O (1.0), MnSO<sub>4</sub>.H<sub>2</sub>O (1.0) and MgSO<sub>4</sub> (0.5). The initial pH was adjusted to 6.0 [11]. After the fermentation, the cells were separated from the medium by centrifugation at 4000 rpm for 10 min (Eppendorf, model 5804R, Germany) and the supernatant was filtered using a 0.45 µm Polyvinylidene Difluoride (PVDF) membrane. The filtered samples (culture filtrate) were used to formulate the bioherbicide applied in the control of *Conyza* sp.

### 2.3. Formulation of bioherbicide.

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 [25]. After the choice of mineral oil as the best oil, a central composite rotatable design (CCRD) with three independent variables (oil concentration, surfactant concentration and hydrophilic-lipophilic balance (HLB)) was evaluated. One assay with only culture filtrate and a control assay with distilled water were also carried out. Table 2 presents the range investigated for each variable. Two surfactants with different hydrophilic-lipophilic balance (HLB) were used: Tween® 80 (HLB = 15.0) and Span® 80 (HLB = 4.3).

The formulations were prepared at 25 °C using a Turrax homogenizer model MA102 (Marconi, Piracicaba, SP, Brazil). Firstly, oil (oil and Span® 80) 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 in Turrax for at 7000 rpm for 10 min. The final emulsion volume was 50 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 *Conyza* sp. was evaluated through post-emergence bioassays with young plants. The plants were collected in the agricultural area of Federal University of Santa Maria (UFSM, Santa Maria, Brazil) and transplanted into polyethylene cups containing 200 g of the commercial substrate (Mecplant®) without treatment. They were cultivated in a greenhouse for 15 days before the application of bioherbicide, which was applied using a backpack sprayer, pressurized by CO<sub>2</sub>, provided by a bar pattern with four tips, model Teejet XR 110.02, with a spacing tips of 0.5 m and pressure of 276 kPa. The travel velocity was 1 m/s and the specific volume of liquid was 200 L/ha. 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 bioherbicide application, following the methodology proposed by Frans and Crowley [26].

### 2.5. Tukey's test.

Statistical differences between the treatments were determined by Tukey's test (p-value < 0.05).

**Table 1.** Herbicidal activity of formulations with different oils.

Treatment	Formulation (mL)	Herbicidal activity (%)
T1	Culture filtrate (47.5) + palm oil (2.5)	10 <sup>A</sup>
T2	Culture filtrate (47.5) + soybean oil (2.5)	10 <sup>A</sup>
T3	Culture filtrate (47.5) + mineral oil (2.5)	80 <sup>B</sup>
T4	Distilled water (47.5) + palm oil (2.5)	5 <sup>A</sup>
T5	Distilled water (47.5) + soybean oil (2.5)	5 <sup>A</sup>

Treatment	Formulation (mL)	Herbicidal activity (%)
T6	Distilled water (47.5) + mineral oil (2.5)	10 <sup>A</sup>
T7	Culture filtrate (50)	5 <sup>A</sup>
T8	Distilled water (50)	0 <sup>A</sup>

\* Means followed by the same letter in the column didn't differ statistically by the Tukey's test at 95% of confidence level (p-value < 0.05)

**Table 2.** Influence of different combinations of adjuvants on the herbicidal activity and the phytotoxicity of the formulations.

Treatment	Oil (% m/v)	Surfactant (% m/v)	HLB	Herbicidal activity (%) <sup>1</sup>	Phytotoxicity (%) <sup>2</sup>
T1	1.81 (-1)	2.8 (-1)	6.5 (-1)	20 <sup>B</sup>	10
T2	4.19 (1)	2.8 (-1)	6.5 (-1)	80 <sup>DE</sup>	10
T3	1.81 (-1)	8.2 (1)	6.5 (-1)	80 <sup>DE</sup>	15
T4	4.19 (1)	8.2 (1)	6.5 (-1)	40 <sup>BC</sup>	10
T5	1.81 (-1)	2.8 (-1)	12.8 (1)	50 <sup>BC</sup>	15
T6	4.19 (1)	2.8 (-1)	12.8 (1)	30 <sup>B</sup>	10
T7	1.81 (-1)	8.2 (1)	12.8 (1)	80 <sup>DE</sup>	10
T8	4.19 (1)	8.2 (1)	12.8 (1)	90 <sup>E</sup>	10
T9	1 (-1.68)	5.5 (0)	9.7 (0)	70 <sup>DE</sup>	10
T10	5 (1.68)	5.5 (0)	9.7 (0)	100 <sup>F</sup>	20
T11	3 (0)	1 (-1.68)	9.7 (0)	40 <sup>BC</sup>	10
T12	3 (0)	10 (1.68)	9.7 (0)	100 <sup>F</sup>	10
T13	3 (0)	5.5 (0)	4.3 (-1.68)	20 <sup>B</sup>	10
T14	3 (0)	5.5 (0)	15 (1.68)	100 <sup>F</sup>	0
T15	3 (0)	5.5 (0)	9.7 (0)	100 <sup>F</sup>	10
T16	3 (0)	5.5 (0)	9.7 (0)	100 <sup>F</sup>	10
T17	3 (0)	5.5 (0)	9.7 (0)	70 <sup>DE</sup>	10
Culture filtrate	0.0	0.0	0.0	40 <sup>BC</sup>	0
Control (distilled water)	0.0	0.0	0.0	0 <sup>A</sup>	0

\* Means followed by the same letter in the column didn't differ statistically by the Tukey's test at 95% of confidence level (p-value < 0.05).

1 Culture filtrate + adjuvants

2 Distilled water + adjuvants

### 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 analyses.

Table 2 presents the results obtained in the 17 formulations of the CCRD, in the formulation with only culture filtrate and in the control assay with distilled water, and the differences between the treatments, determined by Tukey's test (p < 0.05). Treatments T1, T6 and T13 presented slight injury. Moderate herbicidal activity was observed in the treatments T4, T5 and T11. For the treatments, T2, T3, T7 – T9 and T17, a severe injury on plants was reported. In the treatments T10, T12 and T14 – T16, the death of plants was verified. The phytotoxicity results showed a slight wilting at the leaf end in some cases. This behavior could be indicating that the herbicidal activity found in the treatments is associated with the presence of biomolecules produced by *F. fujikuroi* and not to the adjuvants used in the formulations. Comparing the results obtained in the 17 formulations with that one of culture filtrate, it was

possible to observe the intensification of symptoms with the addition of the adjuvants in most treatments. Applying only distilled water to the plants, no symptoms were verified. Figure 1 presents the visual aspects (qualitative description) of *Conyza* sp. plants for all treatments.

The obtained results suggest that the bioherbicide produced by *F. fujikuroi* 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 [27]. This is corroborated by the lower result of herbicidal activity obtained in the treatment T13, which was related to the lowest HLB evaluated (Table 2 and Figure 1). The results obtained were similar to those obtained by Bastos et al. [21], 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 bioherbicide 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 bioherbicide was improved approximately 2.5 times when compared with non-formulated products. Bastos et al. [21] increased 3 times the efficiency of a bioherbicide produced from *Diaphorte* sp. Likewise, Pes et al. [28] accentuated the suppressive effect on the growth of *Conyza* sp. and *Echinochloa* sp. for a bioherbicide formulated from *Diaphorte* sp. Aybeke [29] reported toxic effects of *Fusarium oxysporum* on a root parasitic weed (*Orobanche* spp.). Li et al. [30] 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 fujikuroi* in the control of *Conyza* sp. up to now. Therefore, this work can be considered the first study related to the herbicidal activity of metabolites from *Fusarium fujikuroi* isolated from a local area (Santa Maria, Brazil) in the Pampa biome for the control of this weed.



**Figure 1.** Visual aspect of *Conyza* sp. plants after the application of different formulations: herbicidal activity (culture filtrate + adjuvants: T1 – T17), phytotoxicity (distilled water + adjuvants: T1a – T17a), culture filtrate (F) and distilled water (control test).

## 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 adjuvants 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 3% (w/v) of mineral oil, 5.5% (w/v) of surfactant and HLB of 15, a condition in which a complete death of *Conyza sp.* plants was observed. This promising combination of adjuvants and culture filtrate from *F. fujikuroi* increased 2.5 times the efficiency of bioherbicide for the post-emergence control of *Conyza sp.*

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## Conflicts of Interest

The authors declare no conflict of interest.

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