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Evaluation of aromatic plants hydrosols on the growth of *Trichoderma harzianum*

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ABSTRACT

Objective: To evaluate the compatibility of hydrosols from aromatic plants on the growth of *T. harzianum*.

Design/Methodology/Approach: All hydrosols of *Foeniculum vulgare*, *Plectranthus coleoides*, *Tagetes arenicola*, *T. coronopifolia*, *T. erecta*, and *T. lucida* were evaluated *in vitro* on the mycelium *Trichoderma harzianum*. Their growth rates and inhibition percentages were recorded. *T. harzianum* was inoculated in 50 g of sterilized corn cob enriched with *F. vulgare* hydrosol (3, 5 and 7%), this mixture was incubated at 28±2 °C; spore counting, and viability tests were performed on PDA medium. The Tukey test ($p \leq 0.05$) was used for mean comparisons.

Results: All hydrosols inhibited *in vitro* the mycelial growth of *T. harzianum* at 100%, but at 5% concentration, some hydrosols promoted the growth of the fungus more than the control. The concentration of *F. vulgare* hydrosol influenced the production and viability of *T. harzianum* spores in the cob substrate.

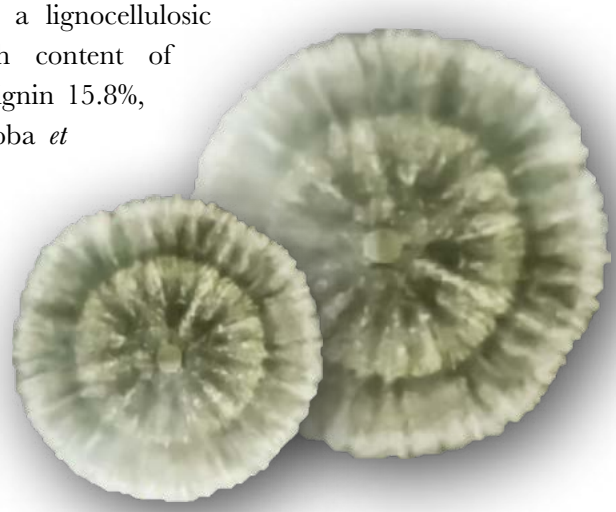
Study Limitations/Implications: This study provides information on the use of hydrosol, which are typically considered waste products.

Findings/Conclusions: At low concentration, *F. vulgare* hydrosol can be used to enrich corn cob and promote the growth of *T. harzianum*.

Keywords: *Trichoderma harzianum*, hydrosol, growth, corn cob.

INTRODUCTION

Due to the harmful effects of synthetic fungicides, there is noticeable interest in the biological activity of organisms such as *Trichoderma harzianum* to control phytopathogenic fungi. *T. harzianum* is characterized by its rapid growth and colonization of substrates (Saravanakumar *et al.*, 2017). Several species of this organism are used as an organic input, marketed in forms such as wettable powder, granules, liquids, and solids added to sorghum or corn straw, cob, cane bagasse, coco peat, rice or corn grains (Bellino & Marroquín, 2015). Cob is a lignocellulosic agricultural by-product with a high content of hemicelluloses (33.6%), cellulose 45%, lignin 15.8%, and particularly xylanases 94% (Córdoba *et al.*, 2013). This represents an advantage, since *Trichoderma* spp. can utilize and degrade lignocellulosic residues composed of cellulose (40-55%), hemicellulose (25-50%), and lignin (10-40%) (la Grange *et al.*, 2010). Depending on the substrate used, the growth and production of spores



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of *T. harzianum* can take 10 to 15 days (Kumar *et al.*, 2014) or 20 to 21 days (Simon, 2011). The evaluation of adjuvants in substrates where *Trichoderma* multiplies could help accelerate the production of fungal biomass and reduce the time needed to obtain it, making the exploration of diverse sources pertinent.

Until now, limited attention has been paid to distillates from aromatic plants as auxiliaries in biotechnological procedures for the reproduction of *Trichoderma*. Although essential oils and hydrosols obtained from hydrodistillation of aromatic species have fungal inhibitory properties (Taglienti *et al.*, 2022), some essential oils do not inhibit the growth of some *Trichoderma* species (Ali *et al.*, 2011; Infante *et al.*, 2013). In the case of hydrosols from aromatic plants, these contain traces of essential oil and are expected to have a biological effect that stimulates mycelial growth (Fiori *et al.*, 2000). Additionally, hydrosols from non-aromatic plant species, such as *Chenopodium*, have been found to favor the growth of fungi such as *Fusarium oxysporum* (Covarrubias *et al.*, 2013).

In general, not enough is known about the effect of hydrosols on fungal growth. Furthermore, in the hydrodistillation processes of aromatic plants, the production of hydrosols, compared to the essential oil, is much higher and this by-product is not always utilized. In Mexico, the families Amaranthaceae, Anacardiaceae, Apiaceae, Asteraceae, Burseraceae, Euphorbiaceae, Fabaceae, Lamiaceae, Lauraceae, Myrtaceae, Piperaceae, Rubiaceae, and Verbenaceae include aromatic species as resource potentially useful in agriculture (Calvo-Irabién, 2018). In the immediate future, experimental evaluation of these species as complementary material for the growth of antagonistic fungi, such as *T. harzianum* (an organism very useful in the control of phytopathogenic fungi), could anticipate a new source of natural adjuvant to stimulate and shorten the production time of *Trichoderma* inoculum. For this reason, in the present study evaluates the compatibility of hydrosols from some aromatic plants in the growth of *T. harzianum*.

MATERIALS AND METHODS

The biological material used included *Plectranthus coleoides* Benth. voucher 22336 (Lamiaceae), *Foeniculum vulgare* Mill. 19561 (Apiaceae), and four species of *Tagetes* (*T. arenicola* Panero & Villaseñor: 35882, *T. coronopifolia* Willd.: 36282, *T. erecta* L.: 35869, and *T. lucida* Cav.: 35872- Asteraceae), these plants have taxonomic support in the Jorge Espinosa Salas Herbarium of the Departamento de Preparatoria Agrícola of the Universidad Autónoma Chapingo, State of Mexico. In October 2022, stems, leaves, and flowers of these plants, grown without fertilization in a greenhouse in Chapingo, Mexico, were subjected to a hydrodistillation process in an Italian-type glass still to obtain hydrosols. The distillation time was 45 min from the beginning of precipitation, the hydrosols were preserved in plastic bottles until use.

In vitro bioassay

Using the poisoned food technique (Balouiri *et al.*, 2016), hydrosol treatments were evaluated, in five replicates per treatment and a control. The experimental unit was a Petri dish with Potato Dextrose Agar (PDA) culture medium and a completely randomized design was used. Each hydrosol was prepared in three concentrations, 5, 50, and 100%.

The flasks containing the treatment substances (hydrosol, PDA, and double-distilled water) were sterilized for 17 minutes in an autoclave at 120 °C and poured into sterile 90 mm glass Petri-dishes.

For this experiment, a strain of *Trichoderma harzianum* kept in the Laboratorio de Resistencia Genética at the Universidad Autónoma de Chapingo, previously identified at the molecular level (accession number MK752565.1 in GeneBank), was used. After 24 hours, a PDA disc with *T. harzianum* inoculum, obtained from a five-day-old colony using a 3.5 mm diameter sterile punch, was placed inverted in the center of the Petri dishes. These dishes were incubated in dark conditions at 28±2 °C in a culture oven. The radial growth of the fungus was measured every 24 hours using a digital vernier, Mycelial inhibition (%I), growth rate (GR), and growth percentages (%G) were calculated with the following formulae:

$$\%I = \frac{D1 - D2}{D1}(100) \qquad GR = \frac{Fd - Id}{Ft - It} \qquad \%C = \frac{C1(100)}{CG}$$

Where: %*I*: percentage of inhibition; *D*1: diameter of mycelial growth of the control (mm); *D*2: diameter of mycelial growth of the influenced (mm); *GR*: growth rate (mm day^{-1}); *Fd*: final growth diameter (mm); *Id*: initial growth diameter (mm); *It*: initial growth time (days); *Ft*: final growth time (days). *C*1: treatment growth (mm), *CG*: control growth (mm).

After carrying out the *in vitro* bioassay, the treatment that promoted the greatest growth of *T. harzianum* was chosen and the second experimental phase, which involved its inoculation in corn cob substrate.

Cob growth

Corn cobs were ground into 1 cm pieces using a forage mill. This substrate was soaked in different concentrations (3, 5 and 7%) of the best hydrosol until it reached a humidity of 55%. The moisture content was estimated on a thermobalance (MA37, Sartorius, United States) and adjusted to pH 6 with 0.2 M citric acid. Then, 50 g of substrate were placed in Erlenmeyer flasks, covered with cotton, and sterilized for 20 min in an autoclave at 120 °C. After cooling to room temperature, inoculation was performed. A suspension of spores (1×10^5) was prepared from a ten-day-old colony of *T. harzianum* and 1×10^5 spores were added to each gram of dry substrate. The flasks were incubated in a culture oven under dark conditions for seven days at 28±2 °C until colonization and sporulation were observed in the control substrate.

Spore count and viability

After eight days, 3 g of sporulated substrate (cob) were weighed and placed in a flask with 10 mL double-distilled water and Tween 20 (0.1 mL L^{-1}). It was then shaken, and the spore count was determined using a Neubauer chamber. This process was repeated three times. To determine viability, one hundred spores were sown in Petri dishes with PDA

medium and incubated at 28±2 °C. The number of germinated colony-forming units (cfu) was counted 48 hours after sowing. This process was repeated four times.

Analysis of data

The recorded data were subjected to Tukey's mean comparison test ($p \leq 0.05$) using SAS Academic Software.

RESULTS AND DISCUSSION

In vitro bioassay

Pure hydrosols inhibited mycelial growth, decreasing the percentage and growth rate of *T. harzianum*. However, at concentrations of 50 and 5%, the percentage and growth rate increased significantly, while the percentage of inhibition decreased (Table 1). The concentration of hydrosols at 5% stimulated mycelial growth similar to the control treatment. Hydrosols contain very small amounts of essential oil, so a high concentration could inhibit mycelial growth (Revilla-Medina *et al.*, 2020). The rapid mycelial growth of *T. harzianum* with some hydrosols at low concentration is likely due to the presence of some non-volatile compounds, such as carbohydrates, which are carried

Table 1. Mean values of growth rate, growth percentage, and inhibition percentage after four days of growth.

Treatments	Growth velocity (mm day $^{-1}$)	Growth (%)	Inhibition (%)
Experimental control (witness)	10.7 ab	100 ab	0 e
P. coleoides 100 %	0.59 e	1 h	99 a
O. coleoides 50 %	5.8 e	38.4 fg	61 b
O. coleoides 5 %	11.8 a	92.6 ab	7 e
T. erecta 100 %	6.08 d	41.4 efg	58 bc
T. erecta 50 %	8.4 bcd	63.5 cd	36 d
T. erecta 5 %	11.0 ab	100.4 ab	0 e
T. coronopifolia 100 %	10.2 abc	62.2 cde	38 d
T. coronopifolia 50 %	10.5 abc	82.1 bc	18 e
T. coronopifolia 5%	11.8 a	98.6 ab	1 e
F. vulgare 100 %	7.7 cd	31.1 g	69 b
F. vulgare 50 %	8.4 bcd	58.2 def	42 cd
F. vulgare 5 %	11.9 a	105.4 a	0 e
T. lucida 100 %	10.4 abc	82.7 bc	17 e
T. lucida 50 %	9.4 abc	93.7 ab	6 e
T. lucida 5 %	11.5 a	104.9 a	0 e
T. arenicola 100 %	8.3 bcd	82.7 bc	17 e
T. arenicola 50 %	10.6 abc	96.7 ab	3 e
T. arenicola 5 %	11.1 ab	100 ab	0 e
CV	13	12	30
MSD	2.91	20	18

CV: Coefficient of variation; MSD: Minimum significant difference.

over during distillation and could serve as nutrients for the organisms (Labadie *et al.*, 2015). The observation that a high concentration inhibits the mycelial growth, and a low concentration stimulates it (Table 1) is consistent with the hormesis phenomenon (Jakobsen *et al.*, 2021).

The 5% *F. vulgare* hydrosol completely covered the Petri-dish by the third day (Figure 1), while the control did so on the fourth day. Due to the rapid growth observed with this hydrosol, concentrations of 3% and 7% were also evaluated. The 7% concentration showed similar growth to the 5% concentration, while the 3% concentration resulted in lower growth (Figure 2).

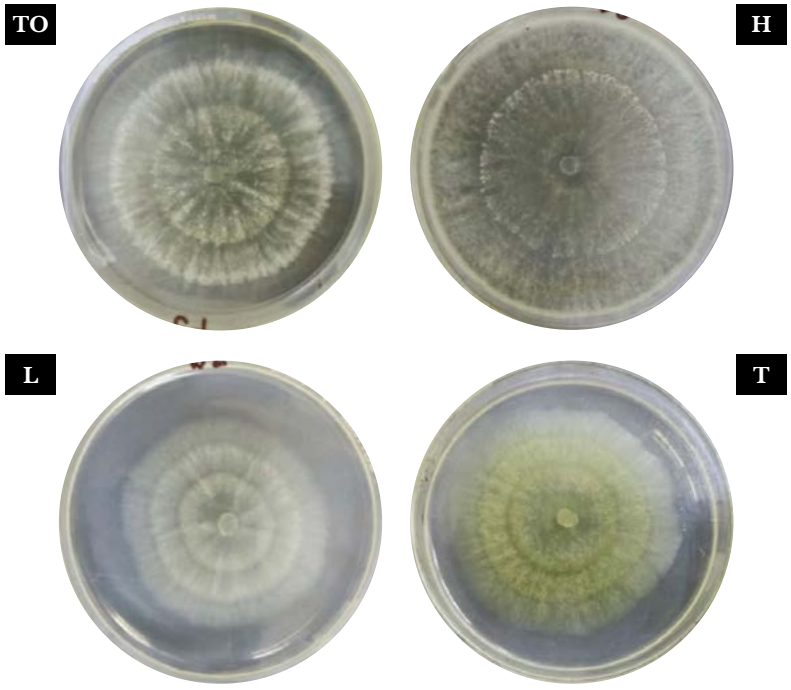

Figure 2. Growth kinetics of *T. harzianum* grown in culture medium (PDA) enriched with *F. vulgare* hydrosol at three concentrations. T0: Witness; H7: *F. vulgare* 7%; H5: *F. vulgare* 5%; H3: *F. vulgare* 3%.

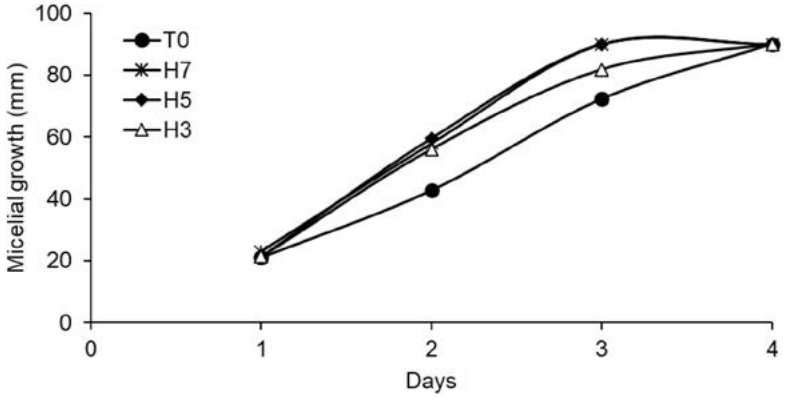

Figure 1. Growth of *T. harzianum* on the third day with 5% hydrosol. TO: Witness, H: *F. vulgare* 5%, L: *Tagetes lucida* 5%, T: *Tagetes erecta* 5%.

Thus far, hydrosols have been used as antifungal (Boyraz & Özcan, 2005) and antiviral agents (Taglienti *et al.*, 2022). However, this study demonstrates their potential to promote the growth of *T. harzianum* is shown. Additionally, due to the inhibition observed at the 100% concentration in the same fungus, further evaluation against phytopathogenic fungi would be promising.

Growth in substrate

The concentration of fennel (*F. vulgare*) hydrosol at 5% was notable for the *in vitro* growth of *T. harzianum* (Table 1). However, when evaluated in corn cob substrate conditions at concentrations of 3, 5, and 7%, only the 3% hydrosol concentration led to greater spore production ($7{,}205\times10^{7}$), compared to the control and the other fennel hydrosol concentrations, over a period of seven days (Table 2). Michel-Aceves *et al.* (2008) obtained 4.43×10^{8} spores mL^{-1} in a period of 21 days using chopped corn cobs. Corn cob contains glucose, xylose, arabinose, galactose, mannose, acetyl groups, lignin, ash, and uronic acid (Da Silva *et al.*, 2015). *T. harzianum*, through its enzymes, converts these hemicelluloses into assimilable sugars (Filho *et al.*, 2017). The hydrosol also influenced the viability of the spores: the lowest concentration (3%) resulted in 70% viability, while the 5% concentration resulted in 56% viability (Table 2). This could be due to the presence of monoterpene alcohols, aldehydes, ketones, and traces of essential oil (Garneau *et al.*, 2014) that can affect spore germination.

Table 2. Production and viability of spores of *T. harzianum* grown on corn cobs enriched with *F. vulgare* hydrosol.

Treatments	Number of spores mL^{-1}	Viability of spores (%)
Witness	3.285×10^{7} b	36 b
F. vulgare 3 %	7.205×10^{7} a	70 a
F. vulgare 5 %	3.3125×10^{7} b	56 ab
F. vulgare 7 %	4.53×10^{7} b	53 ab
CV	12.13	20
MSD	1.45	29

CV: Coefficient of variation; MSD: Minimum significant difference.

This study demonstrates that crushed corn cob enriched with 3% *F. vulgare* hydrosol is suitable for the growth and sporulation of *T. harzianum*. This finding represents an advantage, as it is feasible to use low-cost substrates to achieve higher spore density and reduce cultivation time (Singh and Nautiyal, 2012). Additionally, this enriched substrate can be applied directly to crops or soil (Woo *et al.*, 2014).

CONCLUSIONS

Hydrosols in low concentrations can stimulate the growth of *T. harzianum*. Specifically, *F. vulgare* hydrosol can be used to enrich organic substrate such as corn cob, thereby promote the productivity of *T. harzianum* and reducing the time required for its cultivation.

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