International Journal of Plant Research 2020, 10(1): 11-16
DOI: 10.5923/j.plant.20201001.02

Hexanic Extracts of *Trametes versicolor* (L.:Fr.) Pilát to Control of Tomato Powdery Mildew

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Abstract The powdery mildew on tomato (*Leveillula taurica*) is one of the most important disease of this crop in Mexico, causing losses up to 40% on yield. There are no resistant tomato cultivars, so their control is based on use of chemical fungicides, mainly triazole fungicies. With the objective of obtaining new alternatives to control of this desease, concentrations of 5, 7.5 and 10% (v/v) of Hexanic extracts of *Trametes versicolor* developed on *Luffa aegyptiaca* were evaluated comparing their effectiveness (Abbott) with Amistar Gold® (0.5 L·Ha^{-1}), Serenade Max® (5.0 kg·Ha^{-1}) and a Control. Three applications were made every 7 days and the experimental design used was randomized complete blocks with 4 replicates. The percentage of damaged leaf area was evaluated with an *Exprofeso* scale with 0 to 6 indices 7 days after the first and second application and 7, 14 and 21 days after the third. The results obtained indicate that hexanic extracts offers a control of *L. taurica*, with average control of 47.41 and 57.82% and maximum of 58.16 and 65.09% on concentrations of 7.5 and 10% respectively, suggesting reduce the application interval from 7 to 5 days. The lowest concentration of extracts offered the less control of disease. *L. aegyptiaca* is considered to be an excellent substrate for the development and obtaining of mycelium of *T. versicolor* (R^2: 0.9867). Phytotoxicity symptoms were not detected on the tomato crop cv Cid after applications of Hexanic extracts concentrations

Keywords *Trametes versicolor*, Hexanic extracts, *Leveillula taurica*, *Luffa aegyptiaca*

1. Introduction

Tomato production in Mexico grew at an average annual rate of 3.3% between 2005 and 2015, to reach 3.1 million tons. During that period, the total area allocated decreased at an average annual rate of 3.8%. In 1980, 85,500 ha were sown, in 2000, 75,900 ha and in 2015 the area was 50,596 ha [1].

The decline in the planted area derives from the decrease in the area cultivated in the open sky, while cultivation under protected agricultural conditions continues to expand. The volume of red tomato obtained with the use of these latest technologies increased from 2.9% in 2005 to 32.2% in 2010, and reached 59.6% of the total volume in 2015 [1].

Powdery mildew diseases are caused by fungus that infect leaves, stems, flowers and fruits in nearly 10,000 species of angiosperms [2]. *Leveillula taurica* was identified for the first time Mexico in the state of Sinaloa [3] and can now be detected throughout the country. *L, taurica* is an endoparasite that forms endophytic and epiphytic mycelia with conidiophores branches that grow through stomata [4]. The main symptoms are yellow lesions on the upper surface of the leaf with a dusty-looking sporulation that appears on the underside. In Canada, *L. taurica* infections caused considerable yield losses in greenhouse tomatoes [5], while the field has reported losses of up to 40%, as well as sunburns on fruits due to defoliation severe in the United States [6]. There are no cultivars resistant to this pathogen in the market [7], so the use of fungicides remains the main

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Published online at http://journal.sapub.org/plant

control method. In tomato, it can be controlled by the use of chemical fungicides, biological products or, by acquired systemic resistance (RSA) [8-12].

At present, the use of fungicides such as propiconazole, penconazole, hexaconazole, triadimephon, traidimenol, tridemorf, dinocap and sulfur are the most efficient alternative for the control of powdery mildew [13], as well as new fungicides. generation as the case of the active ingredients belonging to the strobilurin group. The application of inorganic products such as Potassium Silicate (K_2SiO_3) and Monopotassium Phosphate reduce the severity of *L. taurica* in tomatoes by promoting resistance mechanisms [14-16]. The objective of this research was evaluate different concentrations of Hexanic extracts of *Trametes versicolor* as an alternative for the control of *Leveillula taurica* in tomato.

2. Materials and Methods

2.1. Research Location

The production of mycelium and obtaining Hexanic extract of *Trametes versicolor* was carried out in the Pesticide Laboratory of the Postgraduate Program in Plant Protection of the Autonomous University of Chapingo in Chapingo, Mexico; while the field phase was carried out in the Municipality of Coatepec Harinas, State of Mexico, Mexico on tomato cv Cid on flowering stage in greenhouse conditions.

2.2. Mycelium Production of *Tramtes versicolor*

The mycelium used to obtain the extract was obtained from the development of *T. versicolor* strain Mo008 on slices of vegetable sponge (*Luffa aegyptiaca*). 15 slices with average weight of 29.11 g were cut and sterilized under steam pressure at 15 lb by 20 min, subsequently dried at 50 ℃ in an oven and placed in sterile polyurethane containers of 1 L capacity. Each slice was inoculated with 10 dics of 6 mm diameter of Malt-Agar Extract culture medium with *T. versicolor* of 20 days of development, which were randomly placed inside them. The inoculated slices were incubated for 90 days with 12 h of light / dark at 25 ℃ in a BOD-20® incubator. The weight of the container was recorded and subsequently weighed at five-day intervals until the incubation period was completed. The percentage degradation of *L. aegyptiaca* by *T. versicolor* was estimated by equation 1.

2.3. Obtaining the Hexanic Extracts

At 90 days of incubation, 150 g of *L. aegyptiaca* with development of *T. versicolor* were weighed and cut into small pieces and then macerated in a mortar by previously adding liquid nitrogen. The macerate was transferred to a 500 mL ball flask and 250 mL of Hexane was added. The flasks were placed on an oscillator for 48 h. Subsequently, the maceration was filtered and the Hexane was separated from the extract by means of a broken DLab® RE100-Pro steam at 50 ℃ and 100 rpm. Once the solvent was removed, 150 mL of sterile double-distilled water was added and the flask was rinsed to subsequently transfer the extract to 200 mL amber bottles and store at room temperature in total darkness.

2.4. Treatments Evaluated

Concentrations of 5, 7.5 and 10% (v / v) of the *T. versicolor* Hexanic extracts were evaluated, comparing their biological efficacy with the rate of 0.5 L Ha^{-1} of Amistar Gold® SC (azoxystrobin + diphenoconazole [Syngenta®]) and 5.0 kg Ha^{-1} of Serenade Max® (*Bacillus subtillis* strain QST713 [Bayer CropsCience®]) and a Control. An experimental design of randomized complete blocks with four repetitions was used, where each experimental unit consisted of a groove of 1.8 m wide by 5 m of long, equivalent to 9 m^2 by repetition and 36 m^2 by treatment. Three applications were made each seven-day with a pressurized CO_2 equipment with a CD33 full cone nozzle (TeeJet®) with an expense of 633 L Ha^{-1}.

2.5. Evaluation of Biological Effectiveness

An *Exprofeso* diagramatic scale was used to mesuring the level of infection of *Leveillula taurica* on leaflets on greenhouse-grown plants with levels from 0 to 6 (Table 1). The infection of *L. taurica* was recorder at 0 and 7 days after the first and second spray and at 7, 14 and 21 days after the third, randomly sampling 15 leaflets by experimental unit (60 per treatment) of the middle third of the plant. The infection (%) was determined using the Townsend and Heuberger formula (equation 2 [17]). The variance analysis and the Tukey means comparison test with a level of significance of 5% with the SAS® statistical analysis software were applied to the infection percentage data. The control efficacy of each treatment was determinated with equation 3 [18].

Table 1. Diagrammatic scale to differentiate the level of damage of *Leveillula taurica* in tomato leaflets. Coatepec Harinas, State of Mexico. Mexico, 2019

Grade	Description
0	Healthy leaflet
1	Up to 3% of damaged leaf área (DLA)
2	Up to 5% of DLA
3	Up to 10% of DLA
4	Up to 25% of DLA
5	Up to 50% of DLA
6	Up to 75% of DLA

Ex profeso: E. Hernández M. 2019

$$\%D = \frac{[W_i - (W_{fi} - W_e)]}{W_i} * 100 \quad (1)$$

% D: Degradation percentage; Wi = Initial weight of the slice; Wfi = Weight of the slice plus container in the ith evaluation; We = Initial weight of the container

$$\%I = \frac{\sum(n*v)}{CM*N} * 100 \quad (2)$$

% I: Percentage of infection; n: numerical value of each category of the scale; v: number of data within each category; CM: Major category of the scale; N: Sample size

$$\%EB = \frac{(IT-it)}{IT} * 100 \quad (3)$$

% PC: Control or Efficiency in %; IT: Infection in Control; it: Treatment infection

3. Results and Discussion

3.1. Degradation of *Luffa aegyptiaca*

The degradation of the vegetable sponge began after 15 days of inoculation and to 90 days it was degraded by 71.75% on average. The degradation of *L. aegyptiaca* by *T. versicolor* its represented by a non-linear model (Figure 1) with R^2 of 0.9867.

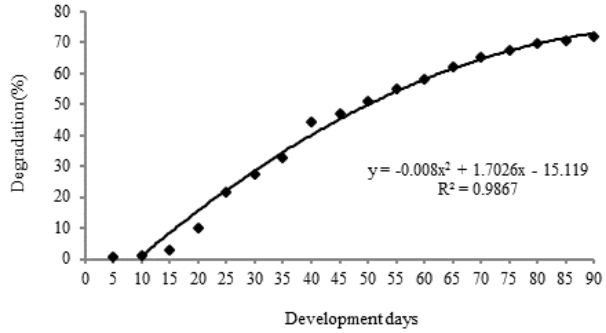


Figure 1. Distribution of the degradation of *Luffa aegyptiaca* slices by *Trametes versicolor*

The degradation process of lignin by *T. versicolor* involves a production process of extracellular ligninolytic enzymes that include lignin peroxidase (LiP) and manganese peroxidase (MnP), which have an oxidizing capacity [19-20]. The degradation of lignin involves a series of reactions that cause the formation of free radicals and result in the breakdown of the molecule [21].

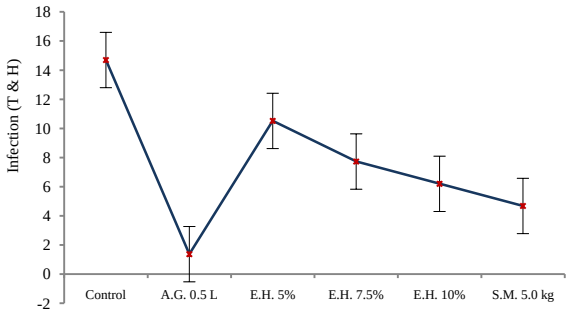
3.2. Biological Efficacy Againts *Leveillula taurica*

The application program began with a mean infection of 1.98%, observing that of the evaluated treatments, the one that presented the lowest infection was the commercial dose of 0.5 L Ha^{-1} of the mixture of azoxystrobin + diphenoconazole (Amistar Gold® SC) as well as the dose of 5.0 kg Ha^{-1} of *Bacillus subtilis* (Serenade Max®), which presented an infection level of 0.79 and 2.86% respectively after three applications (Figure 2).

The concentrations of the Hexanic extracts of *T. versicolor* showed a control effect of *L. taurica* however, as the infection increases in the Control, it also increases in each of the concentrations evaluated. The 10% concentration presented the lowest infection with a range of 3.98 to 9.11%, 21 days after the third application. The 7.5% concentration presented an infection statistically equal to that recorded in the 10% dose in the third, fourth and fifth evaluation and 21 days after third application, both concentrations were statistically equal to the treatment with *B. subtilis* (Table 2). The concentration of 5% (v / v) of the Hexanic extract presented the highest infection in all evaluations carried out.

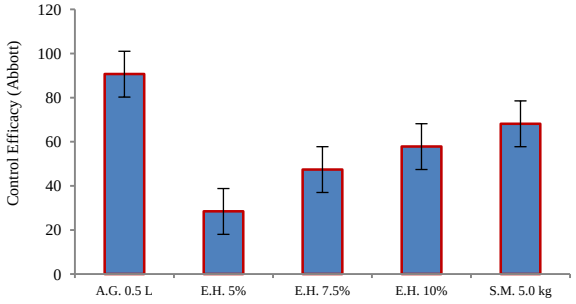
About efficacy (Abbott) of the treatments, it is observed that the Hexanic extracts presented a moderate control of the disease. The concentration of 10% being the best treatment with a control of 65.09% in the second evaluation (Table 3) and this dose maintains the highest control of the pathogen up to 21 days after the third spray with an efficiency of 60.66%, being 2.5% higher than the concentration of 7.5% and 4.34% lower than the dose of 5.0 kg Ha^{-1} of *B. subtilis*. The best treatment was the mixture of azoxystrobin + difenoconazole with a maximum control of 98.62% at 14 days after the second spray. The dose of 5.0 kg Ha^{-1} of *B. subtilis* presented a maximum control of 82.62% after three sprays.

The evaluated concentrations of the Hexanic extract of *T. versicolor* presented average infection of 10.52, 7.73 and 6.2% in the concentrations of 5, 7.5 and 10% respectively, three groups of statistical equality formed by the concentrations of 5 and 7.5%, 7.5 and 10%, and the concentration of 10% and the dose of 5.0 kg Ha^{-1} of *B. subtilis*. The dose of 0.5 L Ha^{-1} of Amistar Gold® SC showed an average infection of 1.37% (Figure 2) and a control efficacy of 90.68% (Figure 3).



A.G.: Amistar Gold® SC; E.H.: Hexane Extract; S.M.: Serenade Max®

Figure 2. Average distribution of Infection (T&H) in the treatments evaluated to control of tomato powdery mildew (*Leveillula taurica*)



A.G.: Amistar Gold® SC; E.H.: Hexane Extract; S.M.: Serenade Max®

Figure 3. Average distribution of Control Efficacy (Abbott) on treatments evaluated to control of tomato powdery mildew (*Leveillula taurica*)

Table 2. Infection (T & H) of *Leveillula taurica* in tomato leaflets cv Cid in the samplings performed. Coatepec Harinas, State of Mexico. Mexico, 2019

			Samplings				
	Treatments	Rates	First	Second	Third	Quarter	Fifth
1.	Control		9.43 e	11.86 d	16.46 e	22.54 e	26.03 d
2.	Amistar Gold® SC	0.5 L Ha^{-1}	1.27 a*	1.27 a*	0.79 a*	0.31 a*	2.70 a*
3.	E. Hexánico	5% (v/v)	6.53 d	11.05 d	12.51 d	14.80 d	16.46 c
4.	E. Hexánico	7.5% (v/v)	4.94 c	7.66 c	8.14 c	12.51 c	10.89 b
5.	E. Hexánico	10% (v/v)	3.98 b c	4.14 b	6.86 c	11.21 c	9.11 b
6.	Serenade Max®	5.0 kg Ha^{-1}	3.02 b	3.66 b	2.86 b	6.05 b	10.24 b

*Treatments with the same letter are statistically equal according to Tukey's test with an $\alpha = 0.05$

Table 3. Efficacy (Abbott) of the treatments evaluated to control of *Leveillula taurica* in leaflets of Tomato cv Cid in the samplings performed. Coatepec Harinas, State of Mexico. Mexico, 2019

			Samplings				
	Treatments	Rates	First	Second	Third	Quarter	Fifth
1.	Testigo absoluto						
2.	Amistar Gold® SC	0.5 L Ha^{-1}	86.53	89.29	95.20	98.62	89.62
3.	E. Hexánico	5% (v/v)	30.75	6.82	23.81	34.33	36.76
4.	E. Hexánico	7.5% (v/v)	47.61	35.41	50.54	44.49	58.16
5.	E. Hexánico	10% (v/v)	57.79	65.09	58.32	50.26	60.66
6.	Serenade Max®	5.0 kg Ha^{-1}	67.97	69.13	82.62	73.15	65.00

*Treatments with the same letter are statistically equal according to Tukey's test with an $\alpha = 0.05$

Hernández et al. [22] evaluated crude extracts of *T. versicolor* to control of a complex of phytopathogenic fungi demonstrating the efficacy of this basidiomycete against *Rhizoctonia solani, Fusarium moniliforme, Botrytis cinerea, Cercospora capsici* and *Alternaria solani,* being the Hexanic extract the most efficient to control these species.

The obtaining of secondary metabolites requires precise research regarding the growth substrate and the solvents involved to obtain it, in this regard Vahidi and Namjoyan [23] evaluated various solvents to obtain extracts of Basidiomycete *Oudemansiella* sp., found that the extract from ethyl acetate it was more efficient to inhibit the germination of *Cladosporium herbarum* and *Aspergillus niger* spores than the extracts whose solvent was chloroform and petroleum ether while Awala and Oyetayo [24] mention that the methanol extracts of *Trametes lactinea* in concentrations of 50 mg / ml are highly effective for the control of *Staphylococcus aureus* and *Aspergillus flavus*.

On the other hand, Arevalo et al. [25] evaluated the antibacterial activity of aqueous and ethanol extracts of *T. versicolor* against ten strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, using the agar diffusion method. 60% of the *P. aeruginosa* strains and 20% of the *S. aureus* strains were sensitive to the aqueous extract, while for the ethanolic extract only 40% of the *P. aeruginosa* strains were sensitive. The value of the Minimum Inhibitory Concentration (MIC) of the aqueous extract for *P. aeruginosa* was 250 mg / ml and for *S. aureus* the value fluctuated between 62.5 to 3.9 mg / ml; the ethanolic extract only had *P. aeruginosa* at 125mg / ml.

A large group of antagonistic secondary metabolites to microorganisms within human and animal health are currently reported and this number is significantly reduced within agriculture, which have been obtained from Basidiomycete extracts. Thus, for example, Hwang et al. [26] isolated the antifungal agent phellinsin A from *Phellinus* sp., capable of inhibiting the citin synthetase I and II of *Saccharomyces crevisaiae* with an IC50 value of 76 µg / ml. Phellinsin A was able to inhibit the development of fungi such as *Colletotrichum lagenarium, Pyricularia oryzae, Rhizoctonia solani, Aspergillus fumigatus* and *Trichophyton mentagrophytes*. Several studies have shown that *Phellinus* species can produce cytotoxic substances [25,27-30], immuno modulators [31-32], antiviral [33], antioxidants and antihepatotoxic [34].

4. Conclusions

The Hexanic extracts of *Trametes versicolor* presented a biological efficacy against the development of *Leveillula taurica* in tomato crop, detecting a direct relationship between the evaluated concentration and its control efficacy (Abbott), exceeding the concentrations of 7.5 and 10%, which they showed an average control of 47.41 and 57.82% respectively after three applications, suggesting to reduce the sprays interval from seven to five days. On the other hand, *Luffa aegyptiaca* is a substrate that favors the development of *T. versicolor* and can be used to the production of mycelium of this Basidiomycete. The tomato crop on which the Hexanic extracts of *T. versicolor* were sprayed at the concentrations evaluated showed no symptoms of phytotoxicity during the development of the investigation.

ACKNOWLEDGEMENTS

To the National Council of Science and Technology (CONACYT) for the support granted to carry out the Postdoctoral Stay, as well as the Master's Program in Plant Protection of the Autonomous University of Chapingo, for the facilities granted to carry out the present investigation.

Dedication

This research is dedicated to my daughters, Sofia Alejandra Hernández Díaz and Cristina Hernández Rubio with all my love (E. Hernández M.)

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