

RESEARCH ARTICLE

ANTISPORULATION ACTION OF TARBUSH PLANT (*FLOURENSIA CERNUA*) TOWARDS CONIDIOSPORES OF PLANT PATHOGENS

Donyo Ganchev*

Department of Chemistry and Phytopharmacy, Faculty of Plant Protection and Agroecology, Agricultural University, Plovdiv, Bulgaria.

*Corresponding author email: donyo@abv.bg

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ABSTRACT

The plant pathogens as *Alternaria solani*, *Monilinia fructigena*, *Botrytis cinerea* and *Venturia inaequalis* cause significant damages on many plants in the European region (particularly in the region of Republic of Bulgaria), especially on orchard cultures which are very important for agricultural industry in this area. There is many existed commercial plant protection products towards this phytopathogens on the market and there is intensive pesticides treatments in order to be overtake infestations and damaging of the plants from this pathogenic fungus. However such king pesticides in the most cases are toxic and harmful for the humans and environment, so there is a need for development and introduction on the pesticide market of the novel environmentally friendly plant protection products against these diseases. In the present research paper an *in vitro* trials were conducted with ethanol extracts from tarbush plant (*Flourensia cernua*) with conidial sporulation of of *Alternaria solani*, *Monilinia fructigena*, *Botrytis cinerea* and *Venturia inaequalis*. The received results show the strong antsporulation action of tarbush plant ethanol extracts towards tested pathogens. However according to the conidiospores of *Alternaria solani*, there was full lack of effectiveness and even slightly stimulation action of germination of spores. This results can be a base for development of the new natural fungicides against tested plant pathogens wicth can be applied as in the commercial agriculture, as in the organic or integrated pest management

KEYWORDS

tarbush plant, *Flourensia cernua*, *Alternaria solani*, *Monilinia fructigena*, *Botrytis cinerea* *Venturia inaequalis*

1. INTRODUCTION

Flourensia cernua is a of flowering plant in the known by the English common names American tarwort and tarbush and the Spanish common names hojasé, hojasén, and hoja ancha (Korthuis, 1988; Manhart, 2011). It is native to the Chihuahuan Desert of North America, where it occurs in the US states of Arizona, New Mexico, and Texas, and the Mexican states of Sonora, Chihuahua, Coahuila, Durango, San Luis Potosí, and Zacatecas (O'Laughlin, 1975). *Flourensia cernua* is a shrub growing from a network of roots that may extend four meters horizontally. This plant is winter-deciduous in most regions, but may retain its leaves in areas with sufficient moisture. In agriculture, this shrub has been studied as a potential supplemental forage for livestock such as sheep (Fredrickson et al., 1994; King et al., 1996). *Flourensia cernua* has medicinal uses. In Mexico it is steeped to make a tea that is consumed to treat various gastrointestinal conditions such as indigestion and diarrhea (Lira-Saldivar et al., 2008).

It is also used for respiratory disorders; its extracts have shown the ability to kill multidrug-resistant *Mycobacterium tuberculosis in vitro* tests (de Rodríguez et al., 2012). Compounds isolated from the plant include flavonoids, sesquiterpenoids, monoterpenoids, acetylenes, p-acetophenones, benzopyrans and benzofurans (Estell et al., 1994; Estell et al., 1996). Extracts of the plant have shown antifungal, anticyanobacterial, and antitermite effects (Tellez et al., 2001). Some studies reveal and their anti-inflammatory and apoptotic effects including antioxidant activity (Salazar et al., 2008; de Rodríguez et al., 2019). Other studies also revel the antifungal activity *in vitro* of *Flourensia spp.* extracts on *Alternaria sp.*, *Rhizoctonia solani*, and *Fusarium oxysporum* and against *Penicillium*

expansum and *Fusarium oxysporum* and *Colletotrichum gloeosporioides* (Guerrero-Rodríguez et al., 2007; Aguilar-Alonso et al., 2013; Apaza and Solis, 2019; de Rodríguez et al., 2007; De León et al., 2013; Prieto et al., 2013).

Other studies show the effectiveness of plant extracts from these native Mexican plants towards *Monilinia fructigena*, *Botrytis cinerea*, anthracnose and *Stemphyllium solani* (Linares Rivero, 2014). The researches show the high potential of the extracts from tarbush to be developed as natural plant protection way against postharvest fungi (Castillo et al., 2012; de Rodríguez et al., 2017; Galván et al., 2014; De León-Zapata et al., 2016; Espinoza Pantigozo, 2016; Rodríguez-Guadarrama et al., 2018; Avila-Sosa et al., 2011). The organic extracts from *Flourensia cernua* express not only antifungal but also bactericidal effects (Molina-Salinas et al., 2006). The plant pathogens as *Alternaria solani*, *Monilinia fructigena*, *Botrytis cinerea* and *Venturia inaequalis* cause significant damages on many plants in the European region (particularly in the region of Republic of Bulgaria), especially on orchard cultures which are very important for agricultural industry in this area.

There is many existed commercial plant protection products towards this phytopathogens on the market and there is intensive pesticides treatments in order to be overtake infestations and damaging of the plants from this pathogenic fungus. However such king pesticides in the most cases are toxic and harmful for the humans and environment, so there is a need for development and introduction on the pesticide market of the novel environmentally friendly plant protection products against these

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diseases (Badillo et al., 2008; Castillo et al., 2011; Ootani et al., 2013; Alvarez-Perez et al., 2020). In the present study an *in vitro* trial of conidiospores of economic important for the region of Bulgaria, plant pathogens were conducted with ethanol extracts from leaves and branches of *Flourensia cernua* as *Alternaria solani*, *Monilinia fructigena*, *Botrytis cinerea* and *Venturia inaequalis*. Due to the many cited in this paper studies about fungicidal activity of tarbush plant extracts, this native Mexican plant is a very promising source of such kind natural plant protection products (Cespedes et al., 2015).

2. MATERIAL AND METHODS

Leaves and branches from tarbush plant (*Flourensia cernua*) were obtained from USDA-ARS, Jornada Experimental Range, Las Cruces, New Mexico, USA. Plant extracts from leaves and branches were prepared by treating 100 g of plant material in 1 liter ethanol in 5-liter dark-colored flask. The mixture stays at room temperature at 20°C, three days, after that shaken for 1 hour at 150 rpm. The extracts were filtered, and the solvent removed under reduced pressure with a rotary evaporator (RVO 004, INGOS Laboratory Instruments “Ltd). The germ tube inhibition tests were conducted in order to be determining ability of the salts to inhibit germination of the conidia of the plant pathogenic fungi. The microscopic slides variety “hanging drop” was sprayed with water solution of tested extracts with desired concentration. After drying of solution, 20 µl conidial suspensions (3*104 spores/ml) was added. The slides were incubated for 24-48 h in thermostat under 22-24°C. Observations with light microscope (10x) were conducted to be determined the germination of the spores (four observation on each slide). The percent of germination was calculated as follows: Percent germinated conidia=number of germinated spores*100 / (number of germinated spores + number of non-germinated spores). According to calculated percents of germination was determined an effectiveness (inhibition) with formula of Abbot (Abbot, 1925). Dose – Response Modeling was performed by R language of statistical computing, drc package (R Core Team, 2020; Ritz et al., 2015).

3. RESULTS

The performed test with conidiospores of *Alteraria solani* show that even 10 % water solution of extracts from leaves and branches of tarbush plant were no able to inhibit the germination. Even more – the extracts slightly stimulate the germination of spores (12 % stimulation for extracts from leaves and 9.4 % % stimulation for extracts from leaves). However, the trials with conidiospores of *Monilinia fructigena*, *Botrytis cinerea* and *Venturia inaequalis* show the strong inhibition potential of the tarbush plant extracts. The next figures show the Dose – Response Models (Curves) of this action.

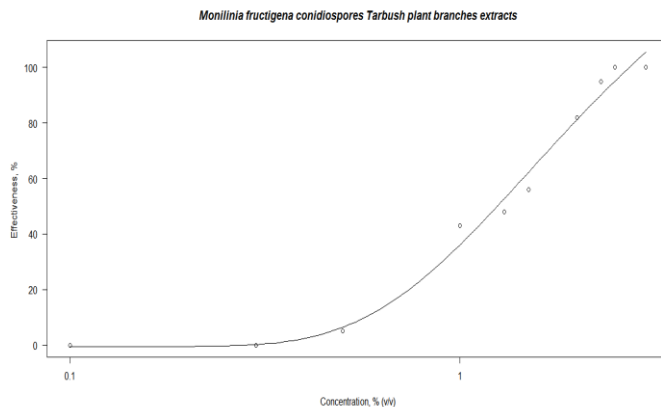


Figure 2: Dose – Response Curve of Tabrbush plant ethanol branches extracts towards conidiospores of *Monilinia fructigena*

- NOAEL = 0.53 %
- LOAEL = 1.11 %
- LD50 = 2.14 %
- LD90 = 2.80 %

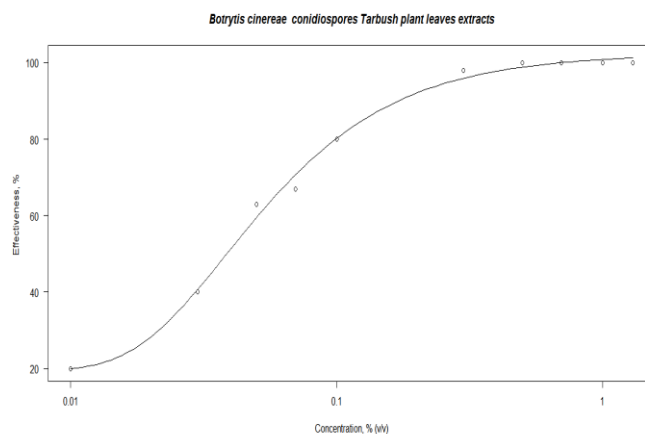


Figure 3: Dose – Response Curve of Tabrbush plant ethanol leaves extracts towards conidiospores of *Botrytis cinerea*

- NOAEL = 0.015 %
- LOAEL = 0.03%
- LD50 = 0.052 %
- LD90 = 0.24 %

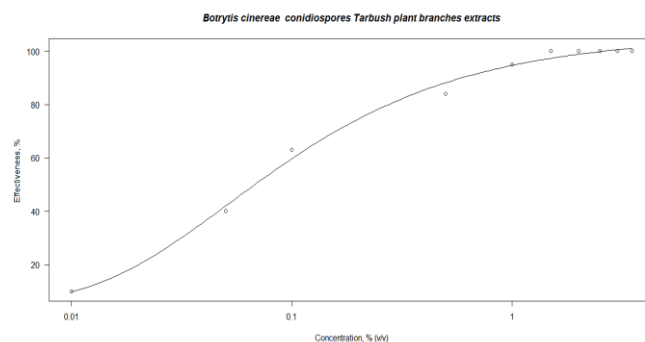


Figure 4: Dose – Response Curve of Tabrbush plant ethanol branches extracts towards conidiospores of *Botrytis cinerea*

- NOAEL = 0.011 %
- LOAEL = 0.032%
- LD50 = 0.085 %
- LD90 = 1.15 %

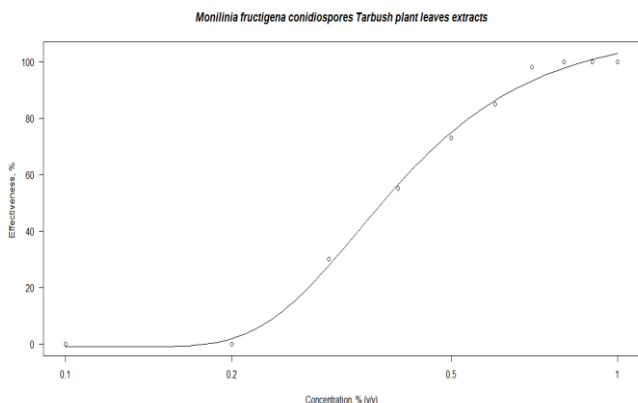


Figure 1: Dose – Response Curve of Tabrbush plant ethonol leaves extracts towards conidiospores of *Monilinia fructigena*

- NOAEL = 0.21 %
- LOAEL = 0.29 %
- LD50 = 0.39 %
- LD90 = 0.84 %

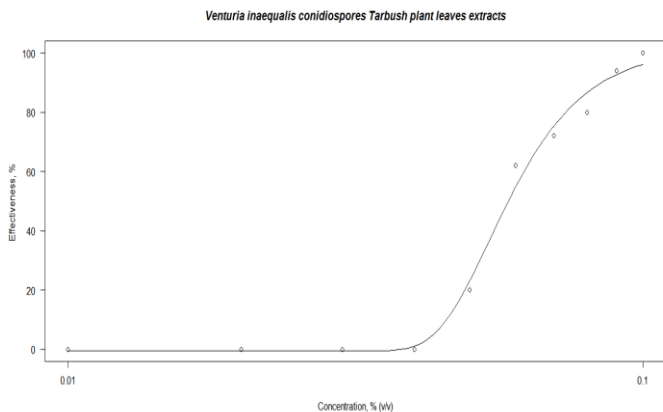


Figure 5: Dose – Response Curve of Tabrbush plant ethanol leaves extracts towards conidiospores of *Venturia inaequalis*

- NOAEL = 0.042 %
- LOAEL = 0.05%
- LD50 = 0.068 %
- LD90 = 0.087 %

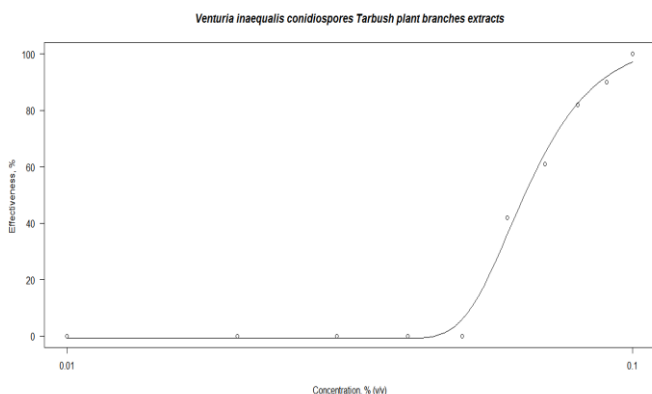


Figure 6: Dose – Response Curve of Tabrbush plant ethanol branches extracts towards conidiospores of *Venturia inaequalis*

- NOAEL = 0.049 %
- LOAEL = 0.056%
- LD50 = 0.078 %
- LD90 = 0.093 %

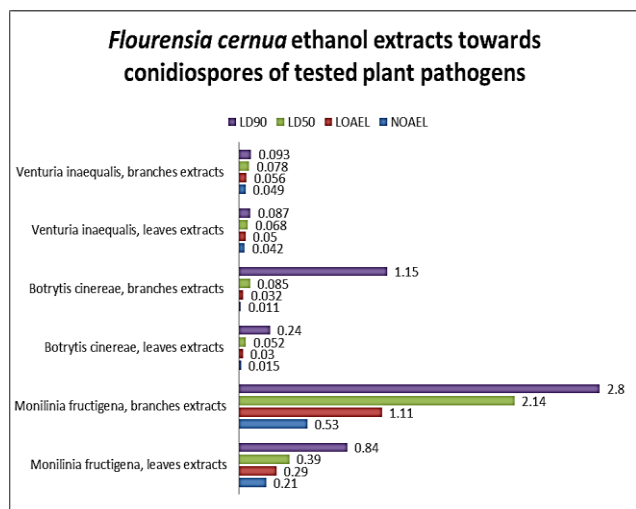


Figure 7: Summarized data received from conducted tests

The Figure 7 show summarized data from the conducted trials. In all of cases the extracts from branches manifest lower effect that extracts from leaves, most significant in the *Monilinia fructigena*. However, in *Venturia*

inaequalis the difference between the effectiveness of extracts from leaves and branches towards germination of conidiospores is minor. From the figure above is obviously that ethanol tarbush plant extracts have the biggest and extremely strong effect towards spores of *Venturia inaequalis*. The effectiveness towards *Monilinia fructigena* is quite low.

4. CONCLUSIONS

Presented results clearly show the strong antifungal and antsporulation of extracts prepared from leaves and branches of tarbush plant (*Flourensia cernua*) confirming from the cited in this paper previous researches with this plant. Surprisingly, the extracts not only do not inhibit the sporulation of *Alteraria solani* but even stimulate germination of conidiospores. Towards other tested plant pathogens however, especially *Venturia inaequalis*, ethanol extracts from tarbush plant (*Flourensia cernua*) show strong inhibition effect. These results can be a base for development of the new natural fungicides against tested plant pathogens which can be applied as in the commercial agriculture, as in the organic or integrated pest management

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