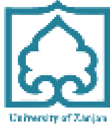

Journal of Medicinal Plants Biotechnology

JMPB

Original Article

Evaluation of the effect of *Thymus vulgaris* oil on growth and pathogenetic mechanisms of *Fusarium oxysporum*

Nima Khaledi

Seed and Plant Certification and Registration Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

ARTICLEINFO

Article history

Submitted: 2020-08-25
Revised: 2020-09-18
Accepted: 2020-11-29

KEYWORDS

Fusarium oxysporum,
Thymus vulgaris,
essential oil,
cellulase.

ABSTRACT

Medicinal plants are potential sources of antimicrobial compounds, which could be used in the management of plant diseases. Research on plant extracts and essential oils which may substitute the use of agrochemical or which may contributes to the development of new compounds is extremely important. Essential oils are promising alternative compounds which have an inhibitory activity on the growth of pathogens. Application of essential oils is a very attractive method for controlling plant diseases. The Fusarium wilt caused by Fusarium oxysporum can be prevented through growing resistant cultivars, soil and seed disinfection by some chemicals. Recently the essential oils as a new method and replace for the control of plant diseases have been accepted. In this study the effect of essential oil of Thymus vulgaris was prepared by using a Clevenger apparatus and its antifungal effect on F. oxysporum was investigated in the laboratory based on mixing essential oil with medium with four replicates. Evaluation of essential oil antifungal was tested at different concentrations of essential oils (i.e. 0-2000 ppm). Growth rate decreases with increasing concentration. No growth was observed at concentrations of more than 1000 ppm. In section survey of enzyme activity, the essential oil reduces cellulase activity but over time, the effect of essential oil decreased. The results of this evaluation of indicate that compounds found in essential oil to reduce fungal growth and enzyme activity. Considering that the production of cellulase enzyme is one of the most important mechanisms influencing penetration to the host, thus reducing the amount of secreted enzymes indicates effect of essential oil on the pathogenic mechanisms and production of cellulase gene expression. These results indicate that essential oils after suitable formulation could be used for the control of Fusarium wilt caused by F. oxysporum pathogen.

* Corresponding author: ***Nima Khaledi***
✉ E-mail: *n_khaledi@areeo.ac.ir*
Journal homepage:

Introduction

Management of seed-borne and soil-borne diseases such as wilt caused by *Fusarium* species has always been problematic. Soil solarization/disinfection, crop rotation and mixed cropping are the best ways of eliminating soil borne pathogens (Sullivan, 2004). Seed treatment with synthetic fungicides such considerably reduce wilt incidence in plant. However, their use is costly as well as environmentally undesirable (Song and Goodman, 2001). The use of resistant varieties is one of the most effective alternative approaches to controlling wilt disease (Singh, 2005). But, due to breakdown of resistance in the face of high pathogenic variability in the pathogen population, the usefulness of many resistant cultivars is restricted to only a few years. Thus there is a need to develop alternative strategies to provide durable resistance over a broad geographic area. In this context, biocontrol is an eco-friendly way of managing fusarium wilt in plant which offers an alternative to fungicides (Prasad and Rangeshwaran, 2000).

Recently, chemical fungicides application has been tried to be replaced by environmentally safe control strategies because of the emergence of fungicide-resistant fungal isolates and public concerns regarding the health and environmental impacts of these chemicals. Essential oil bearing plants constitute a rich source of bioactive chemicals, which

have been reported to have various antifungal properties (Kalemba and Kunicka, 2003). These chemicals are often active against a limited number of species, including the specific target species. They are also biodegradable and non-toxic. Although several essential oils have been reported to have antifungal properties, few have been developed as commercial formulations for use in plant disease control. Plant pathogens attack target cells by producing various cell wall degrading enzyme. With the advent of molecular biology, rigorous research has been carried out on cloning and expression of cellulase genes in various hosts (Lakshminarasimha Reddy and Sreeramulu, 2012). In this study, the effects of *Thymus vulgaris* essential oil on *in vitro* growth and pectinase activity of *F. oxysporum* were examined.

Materials and Methods

Plant pathogenic fungus

Fusarium oxysporum was obtained from seed health laboratory of Seed and Plant Certification and Registration Institute (SPCRI) in Iran. The fungus isolates were maintained on potato dextrose agar (PDA) medium slants at 4˚C, and sub-cultured at monthly intervals.

Plant material and extraction of essential oil

For the extraction of essential oil, leaves of *Thymus vulgaris* were collected September 2013 from Tehran province, Iran. The leaves were washed with

distilled water and dried at room temperature in the shade and away from direct sunlight. Then, using the dried leaves was crushed and plant tissues were passed through of sieve (10 mesh). For isolation of the essential oil, 100 g of dried plant materials were subjected to hydro-distillation for about 3 h, using a Clevenger apparatus. The oil was dried over anhydrous Na_2SO_4 and preserved in sealed glass bottles and protected from the light by wrapping in aluminum foil and stored at 4 °C until used.

Growth inhibition assay in culture medium

The tests were performed using the agar medium assay described by Tatsadjieu et al. (2009) with some modifications. PDA medium with different concentrations of EO (i.e. 0-1000 ppm) were prepared by adding appropriate quantity of EO to melted medium, followed by addition of Tween-20 (100µL to 100 mL of medium) to disperse the oil in the medium. Each Petri-dish was inoculated at the center with a mycelial disc (10 mm diameter) taken at the periphery of *F. oxysporum* colony grown on PDA for 72 h. Positive control (without EO) plates were inoculated following the same procedure. Plates were incubated at 28±1°C for 4 days and the colony diameter was recorded each day. The mycelial growth inhibition (MGI) percentage was calculated according to the following formula: MGI (%) = $[(D_c-D_t)/D_c] \times 100$ Where, D_c = mean diameter of colony in the control (mm) and D_t = mean

diameter of colony in the treatment (mm). Three replicate plates were used per treatment and the experiment was repeated three times.

Effect of EOs on the activity of cellulase

The efficacy of EO obtained from *T. vulgaris* at IC50 concentration in reducing the activity of cellulase enzymes in vitro was determined using the methods described by Khairy et al. (1964) and Abdel-Razik (1970). The production of cellulase enzyme was carried out using a medium containing 4.6 g carboxymethyl cellulase, 5.0 g yeast extract, 5.0 g peptone, and 5.0 g K_2HPO_4 in 1 liter of distilled water and pH 7.2±0.2 as described by MacMillan and Voughin (1964). EO was added to the sterilized medium in each flask to obtain the IC50 concentration. Then, the flask was inoculated with a 1 cm diameter mycelial plug of each fungus. Cellulase activity was determined after 10 days of incubation at 28°C. The supernatants were obtained by filtration and centrifugation at 5000 rpm for 15 min at 4°C. Then, the supernatants were used for crude enzyme preparation. Three flasks were used as replicates for each treatment as well as the control and the experiment was repeated three times.

Cellulase activity was investigated using the method of Wood and Bhat (1988). Carboxymethyl cellulase was used as substrate for cellulase assay as reported in the literature (Han et al., 1995; Deshpande, 1984; Ding et al.,

2001). Briefly, 0.5 ml of cell free supernatant was mixed with 1 ml of 0.7% carboxymethyl cellulase in 0.05 M acetate buffer with pH 4.8 and the reaction mixture was incubated at 50°C for 60 min in static condition. After adding 2 ml of DNS reagent, the mixture was boiled for 10 min at 100°C. The reaction was stopped by adding 1 ml of 40% Potassium sodium tartrate. The absorbance was measured at 550 nm and the amount of reducing sugar released was calculated from the standard curve of glucose. One unit of cellulase activity was defined as the amount of enzyme that catalyzed 1.0 µmol of glucose per minute during the hydrolysis reaction.

Statistical analysis

All experiments were set up in a complete randomized design. The data were analyzed by one-way analysis of variance (ANOVA) and comparison of means was carried out using the Duncan's Multiple Range Test at the level $P \leq 0.05$. Statistical analysis was performed with statistical package for the social sciences (SPSS; version 21) softwares.

Results

Antifungal activity of essential oil on mycelial growth

The effects of different concentrations of the EO on mycelial growth of *F. oxysporum* are shown in Fig. 1. The EO inhibited the growth of *F. oxysporum* in a dose dependent manner. Complete

growth inhibition was observed at concentrations higher than 1000 ppm.

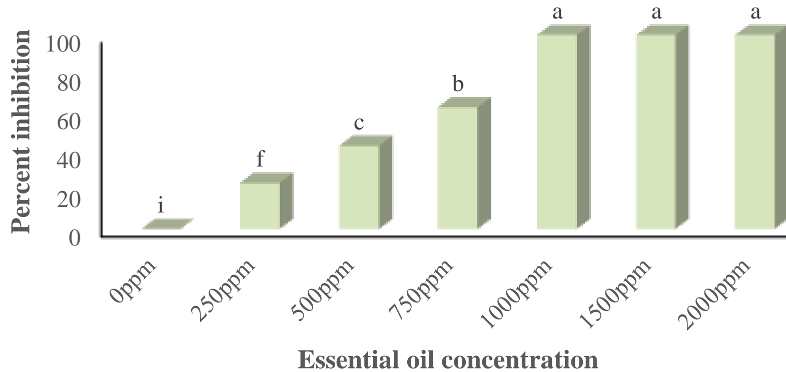

Fig. 1. Effect of different concentrations of *T. vulgaris* essential oil on mycelial growth of *F. oxysporum*

Effect of essential oil on cellulase activity of *F. oxysporum*

The effects of different concentrations of the essential oil on cellulase activity of *F. oxysporum* are shown in Fig. 2. In vitro, *F. oxysporum* showed maximum cellulase activity after 168 hours post-culturing on liquid medium (hpc), respectively, and decreased afterward. The present data revealed significant decrease in cellulase activity of all treatments having essential oil compared to control. So, the decrease in cellulase activity observed in this research may reflect a process elaborated of effective compounds of essential oil to reducing pathogenic. Cellulolytic enzymes serve as invasive agents that enable the pathogen to penetrate the tissue of its host or as digestive agents that enable plant tissues to be penetrated by other enzymes and

enable cellulase itself to be utilized as a carbon source (Olutiola and Cole, 1976). *F. oxysporum* is necrotrophic phytopathogen which enter the host tissue either by exerting mechanical pressure from the growing hyphae or by dissolving the cell wall through CWDEs. Similarly, a previous report indicated that aqueous extracts of various plant species reduced the mycelial growth of *R. solani* and *Fusarium solani* and significantly inhibited polygalactronase and cellulolytic activity of these fungi *in vitro* (Abd-El-Khair and El-Gamal Nadia 2011). In conclusion, essential oil of *T. vulgaris* could be applied as an alternative to synthetic fungicides for the control of *F. oxysporum.*

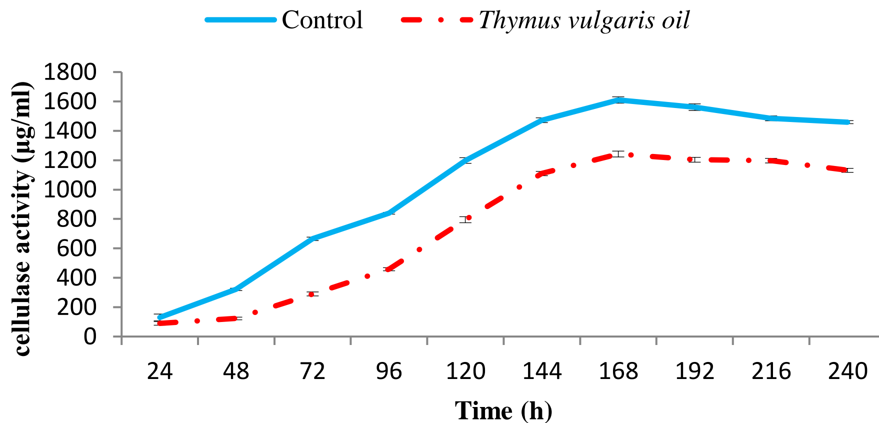

Fig. 2. Effect of *T. vulgaris* essential oil on cellulase activity of *F. oxysporum*

Sponsor: This research has not received any financial support from funding organizations.
Conflict of interest: According to the author's statement, this article has no conflict of interest.
Derived from thesis/dissertation: This article is not derived from thesis/dissertation.

References

- Abd-El-Khair H, El-Gamal Nadia G, (2011) "Effects of aqueous extracts of some plant species against *Fusarium solani* and *Rhizoctonia solani* in *Phaseolus vulgaris* plants" Archives of phytopathology and plant protection, 44(1):1-16.

- Abdel-Razik A.A, (1970) "The parasitism of white *Sclerotium cepivorum* Berk., the incitant of white rot of onion" PhD thesis, Faculty of Agriculture, Assiut University.

- Deshpande M.V, Eriksson K.E, (1984) "Reutilization of enzymes for saccharification of lignocellulosic materials" Enzyme and Microbial Technology, 6:338-340.

- Ding S.J, Ge W, Burevell J.A, (2001) "Endoglucanase I from the edible straw mushroom, *Volvariella volvacea*, purification, characterization, cloning and expressions" European Journal of Biochemistry, 268:5687-5695

- Han S.J, Yoo Y.J, Kangs H.,. (1995) "Characterization of a bitunctional ceilulase and its structurai gene" Journal of Biological Chemistry, 270: 6012-26019.

- Haware M.P, Kannaiyan J, (1992) "Seed transmission of *Fusarium udum* in pigeonpea and its control by seed-treatment fungicides" Seed Science Technology, 20: 597-601.

- Kalemba D, Kunicka A, (2003) "Antibacterial and antifungal properties of

essential oils" Current Medicinal Chemistry, 10:813-829.

- Khairy E.M, Sammour H.M, Ragheb A, Ghandour M.F, Aziz K, (1964) "A Laboratory Manual of Practical Chemistry" Cairo, Egypt: Dar El-Nahda El-Arabia. 1-142.

- Lakshminarasimha Reddy P, Sreeramulu A, (2012) "Isolation, identification and screening of pectinolytic fungi from different soil samples of chittoor district" International Journal of Life science and Pharma Research, 186-193.

- MacMillan JD, Voughin RH, (1964) "Purification and properties of a polyglacturonic acid- transeliminase produced by *Clastridium multiformentans*" Biochemistry, 3:564-572.

- Miller G.L, (1959) "Use of dinitrosalicylic acid reagent for determination of reducing sugar" Analytical Chemistry, 31,426.

- Olutiola P.O, Cole O.O, (1976) "Production of a cellulase complex in culture filtrates of *Aspergillus tamarii* associated with mouldy cocoa beans in Nigeria" Plant Physiology, 37:313-316.

- Prasad R.D, Rangeshwaran R, (2000) "Effect of soil application of a granular formulation of *Trichoderma harzianum* on *Rhizoctonia solani* incited seed rot and damping-off of chickpea" Journal of Mycology and Plant Pathology, 30(2):216-220.

- Singh R.S, (2005) "Plant diseases. New Delhi: Oxford

and IBH 547 publication" Co. Pvt. Ltd. P. 470-476.

- Song F, Goodman R.M, (2001) "Molecular biology of disease resistance in rice" Physiology & Molecular Plant Pathology, 59:1-11.

- Sullivan P, (2004) "Sustainable management of soil-borne plant disease-soil systems guide" National sustainable agriculture information service. Fayetteville, Arkansas.

- Tatsadjieu N.L, Jazet Dongmo P.M, Ngassoum M.B, Etoa F-X, Mbofung C.M.F, (2009) "Investigations on the essential oil of *Lippia rugosa* from Cameroon for its potential use as antifungal agent against *Aspergillus flavus*" Food Control, 20:161-166.

- Wood T.M, Bhat M, (1998) "Methods for measuring cellulase activities" Methods in Enzymology, 160: 87-112.