ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 02, pp.26580-26583, February, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

THE EFFECT OF FOUR MEDICINAL PLANT EXTRACTS ON THE GROWTH OF TOMATO WILT CAUSING FUNGI *FUSARIUM OXYSPORUM* F.SP. LYCOPERSICI

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ARTICLE INFO

Article History:
Received 17th November, 2015
Received in revised form
08th December, 2015
Accepted 20th January, 2016
Published online 27th February, 2016

Key words:

Antifungal potential,
Fusarium oxysporum,
oisoned food technique,
Plant extracts.

ABSTRACT

Biological control of soil borne plant pathogens is a potential alternative to agrochemicals that are harmful to human health and for the environment. Antifungal potential of acetone extracts of four medicinal plants (Acorus calamus, Arctium lappa, Origanum vulgare and Thymus serpyllum) of different families were tested against Fusarium oxysporum f.sp. lycopersici causal agent of wilting in Tomato. Leaves of four medicinal plants were examined for antifungal activity in vitro using the poisoned food technique. Among four plant species tested, acetone extract of Arctium lappa has recorded significant antifungal activity against the test fungi. Whereas, other plant extracts showed moderate to minimum antifungal activity. These findings suggests that some plant extracts tested possess antifungal activities against Fusarium oxysporum and could be used as potential antifungal agents for the control of fungal plant diseases.

Citation: **Pratibha Rawal, Rajendra Singh Adhikari and Asha Tiwari, 2016.** "The Effect of four Medicinal Plant Extracts on the Growth of Tomato Wilt Causing Fungi Fusarium oxysporum f.sp. lycopersici", *International Journal of Current Research,* 8, (02), 26580-26583.

INTRODUCTION

Fungi are potent destroyers of foodstuffs, making them not only unfit for human consumption but also decreases their nutritive value as well by producing certain mycotoxins (Marin *et al.*, 1999; Janardhana *et al.*, 1998). Genus *Fusarium* and its species are examples of phytopathogenic and toxin producing fungi that have been reported to be widespread throughout the world, which can cause serious health problems. *Fusarium* is a soil born and plant pathogenic fungus, and are responsible for destroying crops and dramatically reducing production yields (Matos and Ricardo, 2006: Agrios, 2005). The Fusarium wilt of tomato (*Lycopersicion esculantum* Mill) caused by Fusarium oxysporum f. sp. *lycopersici* (Snyder and Hansen). It (Fol) is recognized as a devastating disease in tomato growing areas all over the world (Beckman, 1987; Bondad-Reantaso *et al.*, 2005) also in different regions of India from severe to moderate (50-60%) percentage (Sherf and Macnab, 1986; Jiskani *et al.*, 2007; Chakraborty and Chatterjee, 2009).

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Tomato (*Lycopersicon esculentum*), is one of the most important vegetable in many countries has a worldwide economic and nutritive importance (Khoso, 1994). *Fusarium oxysporum* f.sp. *lycopersici* is a soil isolated pathogen in the class Hyphomycetes that causes wilting in tomato as the only host of pathogen (Rai *et al.,* 2011). *Rhizoctonia solani* and *Fusarium oxysporum* are major soil isolated fungal pathogens known to cause great harm to the both greenhouse and field grown tomatoes in the warm vegetable growing areas of the world (Sneh *et al.,* 1986). *Fusarium oxysporum* infected the roots mainly through penetrating wounds and proceeds into and throughout the vascular system, leading to functional collapse, systemic wilting due to xylem clogging and often the death of the infected (wilted) plant (Bowers *et al.,* 2000).

In India, Kanpur (U. P.), the vegetable cultivators and farmers suffer more than 25.14-47.94% crop damage due to *Fusarium* wilt of tomato. Singh and Kamal (2012), reported higher 10-90% loss in yield of tomato crop in temperate tomato growing regions due to this disease. All over the world, the use of medicinal plants has significantly helpful for primary health care (Maciel *et al.,* 2002). Plants possess antimicrobial properties because of the presence a wide variety of secondary metabolites, such as tannins, terpenoids,

alkaloids, flavonoids etc. Microbiologists have strong reasons to be interested in the antimicrobial properties of plant extracts and their scopes in medical science.

MATERIALS AND METHODS

Collection of Plant Material

Fresh disease free leaves of four medicinal plant species as, *Acorus calamus* (Acoraceae), *Arctium lappa* (Asteraceae), *Origanum vulgare* and *Thymus serpyllum* (Lamiaceae) were collected from Pithoragarh district, Uttarakhand (India). Selected four medicinal plants were locally available in sufficient quantities.

Preparation of Extracts

Solvent Acetone extract

Leaf samples of 20 gm of all plants were washed thoroughly with sodium hypochlorite solution and finally with sterile distilled water, air dried and then ground with the help of sterile pestle and mortar in 100ml acetone solvent to make a ratio of 1:5 (Material:Solvent). Extracts were filtered through double layered Whatman No. 1 filter paper and heat sterilized in an autoclave at 121 °C for 30 min. Extracts were stored aseptically in airtight bottles and served as mother extract.

Antifungal Activity Assay of Acetone extract by Poisoined Food Technique (Sangvikar *et al.*, 2012)

PDA medium with solvent extracts of the test plants were prepared and autoclaved and poured into pre sterilized petriplates (20 ml PDA+ 1ml plant extract = 21ml each) and allowed to solidify. After complete solidification of the medium, five mm disc of seven day old culture of the test fungi were placed aseptically in the centre of the petriplates and incubated at 28± 2°C for six days and observation were taken on seventh day. The colony diameter was recorded in millimeters. PDA medium devoid of any extract served as control (Minz *et al.*, 2012). Each treatment was performed four times. The fungitoxicity of extracts was calculated in terms of percent inhibition of mycelia growth by using the formula:

% inhibition = dc- dt / dc x 100 Where,
dc= Average increase in mycelia growth in control.
dt = Average increase in mycelia growth in treatment (Singh and Tripathi 1999). Experiments were performed in the laboratory of L.S.M.G.P.G. college Pithoragarh.

Data analysis

Data from antifungal activity screening were analyzed using simple statistics from Microsoft Excel and recorded in appropriate tables as mean ± standard deviation of mean.

Table 1. Antifungal Assay of Plant Extracts by Poisoned Food technique

Technique S.No.	Plants Name (Acetone extarct)	Average radial growth in Control Treatment (mm)	Average radial growth in acetone extract Treatment (mm)	% Inhibition of Mycelial Growth
1	Acorus calamus	72.50 ± 6.4	24.00 ± 6.4	66.89%
2	Arctium lappa	74.25 ± 6.1	10.75 ±1.1	85.52%
3	Origanum vulgare	62.50 ± 2.0	39.75 ± 4.1	36.40%
4	Thymus serpyllum	62.75 ± 2.2	37.25 ± 5.5	40.63%

Results are the mean of four replicants ± S.D.

Acorus calamus

Arctium lappa

Test Fungi: Microorganism used in this study *Fusarium oxysporum* f.sp. *lycopersici* was obtained from Microbial Type Culture Collection MTCC, Institute of microbial technology, Chandigarh. Fungus culture (MTCC 1755) was maintained in Potato Sucrose Agar medium for an optimum pH of 6.8.

RESULTS

The present study revealed that acetone extracts had a higher fungitoxic activity against *Fusarium oxysporum* f. sp. *lycopersici*.

Origanum vulgare

Thymus serpyllum

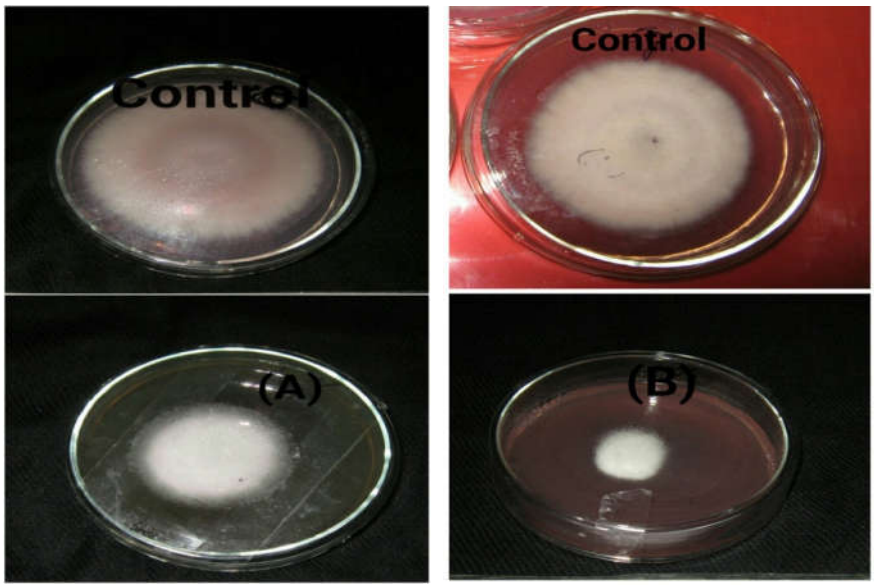


Plate-(A). ***Acorus calamus*** **extract treatment, Plate-(B).** ***Arctium lappa*** **extract treatment**

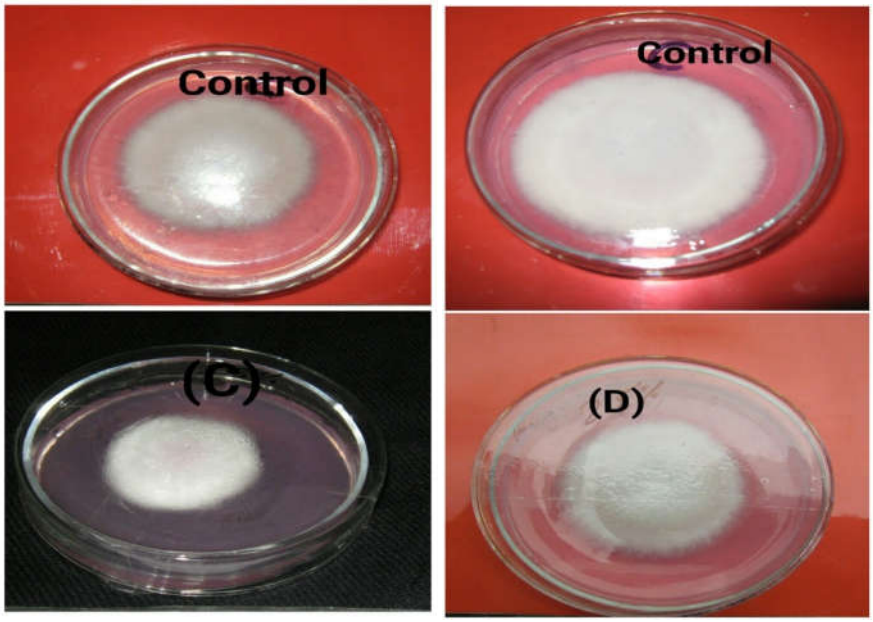
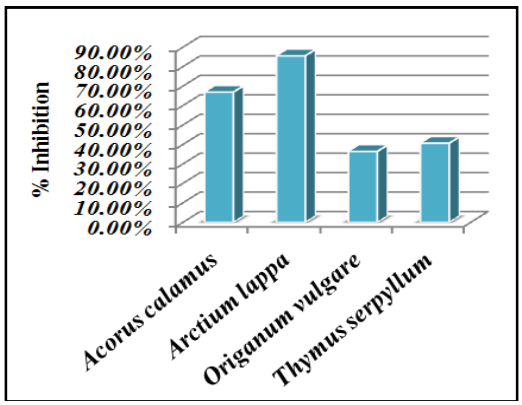


Plate-(C). ***Origanum vulgare*** **extract treatment, Plate-(D).** ***Thymus serpyllum*** **extract treatment**
C=Control Treatment

Figure 1. Antifungal activity of leaf extracts of selected plants in acetone solvents against ***Fusarium oxysporum*** **f.sp.** ***lycopersici*** **by poisoned food method**



Graph 1. Antifungal activity of leaf extracts of selected plants in acetone solvents against *Fusarium oxysporum* f.sp. *lycopersici* by poisoned food method

From Table 1 and Figure 1 it is clear that *Arctium lappa* was highest effective with 85.52% inhibition over mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*. However in case *Acorus calamus, Origanum vulgare and Thymus serpyllum* the percent inhibition was recorded to be as 66.89%, 36.40 and 40.63 respectively.

DISCUSSION

The development of microbial resistance to the available antibiotics has informed the need to discover more natural disease control options which has further led to investigate antimicrobial activity of some medicinal plants (Prakash *et al* 2012; Singh *et al.,* 2012). Studies have been carried out so far to explore useful antibacterial and antifungal compounds from plants (Sofowara, 1993; Valsaraj *et al.,* 1997; Perumalsamy *et al.,* 1999). Weideman (2005) reported acetone extracted the highest concentration of plant material on average, for all the medicinal plants investigated. This finding is also supported by Martini and Eloff (1998), that reported on acetone that extracted the most complex mixture of different compounds. Therefore this study suggests these plant extracts would be helpful in treating wilting disease in field.

Conclusion

The results of this study suggest a fairly good correlation between medicinal use and the *in vitro* antifungal activity. It has been concluded from present research that certain plant extracts are a source of cheap and effective fungicides of *Fusarium oxysporum* f.sp. *lycopersici*, also it doesn't have human and environment health implications.

These findings gave an idea that the plant extracts may be used as potential alternatives to synthetic fungicides for seed treatment to protect them against seed and soil borne pathogens.

Acknowledgment

Author is thankful to Dr. R. S. Adhikari, Associate Professor and Head of Botany Department, L.S.M. Govt. P.G. College Pithoragarh, Uttarakhand, for giving an opportunity to work under their inspiring guidance and for providing necessary support during research work.

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