

REMEDIATION OF TOMATO (Lycopersicon esculentum) FRUIT ROT CAUSED BY Fusarium oxysporum f. sp. lycopersici USING VARIOUS PLANT EXTRACTS

NOWSHEEN HASSAN¹ & HALEEMA ASHRAF²

¹Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar- Srinagar Jammu and Kashmir, India

²Assistant Professor, Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar- Srinagar Jammu and Kashmir, India

ABSTRACT

The antifungal activity of five plant extracts *viz.*, *Allium sativum*, *Zingiber officinale*, *Allium cepa*, *Mentha spicata* and *Curcuma longa* were evaluated against the tomato phytopathogenic fungi, *Fusarium oxysporum* f. sp. *Lycopersici* by Poison Food Technique. Results showed that all the aqueous plant extracts tested *in vitro* were found significantly effective in reducing the percentage mycelial growth of *F. oxysporum* f. sp. *lycopersici* over untreated control. However plant extract(@ 10,20,30,40 and 50%) of *A. sativum* recorded lowest mean colony diameter of 38.96 mm and highest mean mycelial growth inhibition (39.12%) followed by *Z. officinale* and *A. cepa* which recorded mean mycelial growth of 40.80mm and 44.46mm and mean mycelial growth inhibition of 37.70% and 31.38% respectively. *C. longa* was lowest inhibitory (20.52%) against *F. oxysporum* f. sp. *lycopersici* out of all the test plants.

KEYWORDS: Remediation, Fruit Rot, Plant Extracts, Tomato

INTRODUCTION

Tomato is attacked by variety of pathogens; predominant being the fungal fruit rots (Nisa *et al.*, 2010). These fungal rots are responsible for causing serious production problems that therefore become menace for successful cultivation. Fungal rot is world-wide problem as it has been reported almost in all parts of the world. According to Sokhi and Sohi (1982), the destructive pathogen causing fruit rots on tomato is reported from the countries where moisture is plentiful and temperatures are moderate, favouring their development. Losses due to fruit rot diseases are associated with a number of factors such as commodity type, cultivar susceptibility to the disease, the environmental events (temperature, relative humidity, atmosphere composition etc.) and/or ripeness stages. Therefore, treatments and handling methods for the control of diseases are required to prevent losses. Consumer's increasing desire for high quality and nutritional foods has created a need for longer market keeping-period for both domestic and export markets. This is especially true for tomatoes which ranks number one among vegetables, contributing vitamins and minerals availability to consumers (Rick, 1978).

In an era of sustainable agriculture, it becomes obligatory to avoid the use of synthetic chemicals. Therefore, bio-control techniques need to be evaluated. Plant-derived substances have recently become the matter of great interest owing to their versatile applications (Baris *et al.*, 2006). They are not only easy to prepare at low price but also non polluting toxins as compared to commercial fungicides. There are some reports of using plant extracts as bio-preservatives to enhance the shelf life of tomatoes during storage. The extracts of many herbal plants have shown antimicrobial activity (Sheik and Agnihotri, 1972) and rot of tomatoes during storage (Hasabins and D'Souza, 1988). Therefore the present study

was undertaken to investigate the antifungal activity of aqueous extracts of five different plants *viz.*, *A.sativum*, *A.cepa*, *C.longa*, *M. spicata* and *Z. officinale* at different concentrations against the pathogen *F.oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

Preparation of the Plant Extracts

The standard stock solutions of plant extracts were prepared with different concentrations (10, 20, 30, 40 and 50%) separately in aqueous solventas per the procedure given by Sindhan *et al.* (1999). Botanicals and their parts used for the preparation of extracts which were obtained from local markets are given.

Aqueous Extract

Aqueous plant extracts were prepared from different plant parts in pestle and mortar by washing with tap water followed by sterile water and then crushed in pestle and mortar in sterile distilled water at the rate of one gram air dried in 1ml of water (1:1 w/v). The pulverized mass was squeezed through four folds of muslin cloth and finally through Whatman filter paper (No.1). This was the standard solution (100%) of plant extract and the same solution was diluted with distilled water to desired concentrations.

Botanical name	Local name	English name	Family	Plant part used	
Allium cepa	Ganda	Onion	Amaryllidaceae	Bulb	
Allium sativum	Rohan	Garlic	Amaryllidaceae	Bulb	
Curcuma longa	Haldi	Turmeric	Zingiberaceae	Rhizome	
Mentha spicata	Pudina	Mint	Labiatae	Leaves	
Zingiber officinale	Ardrak	Ginger	Zingiberaceae	Rhizome	

Determination of Antifungal Activity of Plant Extracts On Fruit Rot Pathogens of Tomato by Poison Food Technique

The Poison Food Technique (Grover and Moore, 1962) was followed to evaluate the efficacy of botanicals in laboratory against fruit rot pathogen at different concentrations (10, 20, 30, 40, 50%) and control (without plant extracts) of botanicals with three replications.

Poison Food Technique

Five days old fungal culture was punched aseptically with a sterile cork borer of generally 7 mm diameter. The fungal discs were then put on the gelled agar plate. The agar plates were prepared by impregnating desired concentration of plant extract at ambient temperature. The plates were incubated in BOD incubator (Narang Scientific Works, NSW Pvt. Ltd. GI-111, Mayapuri, New Delhi) at temperature 26±1°C. Colony diameter was recorded. Percentage inhibition of mycelial growth was evaluated by comparing the colony diameter of poisoned plate (with plant extract) and non-poisoned plate (without plant extract) and calculated using the formula:

% Mycelial inhibition =	Mycelial growth (C) – Mycelial growth (T)	——————————————————————————————————————
	Mycelial growth (C)	

Where,

(C) = Control

(T) = Treatment

RESULTS

Effect of Aqueous Extract

The aqueous extract of the test plants evaluated for their inhibitory effects on growth of *F. oxysporum* f. sp. *lycopersici* (Table 1 and Figure 1-2) exhibited significant inhibitory effects. On an average, the aqueous extract of *A. sativum* exhibited maximum inhibition (39.12%) of *F. oxysporum* f. sp. *lycopersici* followed by *Z. officinale* and *A. cepa* which showed inhibition of 37.70 and 31.38 per cent respectively. *C. longa* was lowest inhibitory (20.52%) against *F. oxysporum* f. sp. *lycopersici* out of all the test plants. However all test plants provided significant growth inhibition compared with untreated control. The treatment concentrations exerted statistically significant differences. Inhibition (46.32%) was recorded at highest treatment concentration of 50 per cent followed by 40, 30 and 20 per cent concentrations respectively, whereas minimum average inhibition (17.90%) was recorded at lowest treatment concentration (10%). A significant interaction between test plants and their aqueous treatment concentrations also existed. At 50 per cent concentration was next best providing growth inhibition of 52.82 per cent followed by *M. spicata* and *A. cepa* with growth inhibition of 42.22 and 41.44 per cent, respectively. At 10 per cent concentration *C. longa* provided lowest inhibition of 11.70 per cent.

DISCUSSION

The aqueous extract of *A. sativum* exhibited maximum inhibition (39.12%) of *F. oxysporum* f. sp. *lycopersici*. Sahavaraj *et al.*, 2006 also reported that *A. sativum* was known to have anti-fungal activity. Nisa *et al.* (2011) reported also that the extract of *A. sativum* at highest concentration was found to be most effective in reducing the spore germination followed by highest concentration of extract of *A. cepa* and *M. arvensis*. Similar results were found by Misra and Dixit (1976). The results are also supported by the findings of Bowers and Locke (2000) who also reported the maximum inhibition in spore germination of *Fusarium* sp. was exhibited by *A. sativum*. Jacob and Siva Prakasan (1994) and Arya *et al.* (1995) studied the antifungal activity of the extracts of various plant species against *Fusarium pallidoroseum* and reported inhibitory effect of extracts of garlic bulbs and *Bignonia* leaves on the mycelial growth of *Fusarium pallidoroseum*. Datar (1999) and Anwar and Khan (2001) observed the same results with the plant extracts of other plants. Surekha *et al.* (2010) reported that *A. sativum* extract proved to be more efficient in controlling spoilage microorganisms, causing enhancement of shelf life and reducing physiological weight loss.

The variation in the growth of the fungi in extract of different plants is because of the differences in qualitative and quantitative toxic/stimulatory components to the fungus. Also the differences in the potentials between plant extracts may be attributed to the susceptibility of each fungal pathogen to different extract concentrations. This is supported with the results of Okigbo and Nmeka (2005).

The inhibitory potential of plant extracts may be attributed to the phytochemical compounds like phenols,

phenolic acids, quinones, tannins, flavonoids, flavones, flavonols and coumarins. These groups of compounds exhibit antimicrobial effect and serve as plant defence mechanisms against pathogenic microorganisms. Phenolic and flavonoid compounds are important due to their ability to serve as antioxidants. Many phenolic compounds have been reported to possess potent antioxidant activity and anti-carcinogenic, anti-microbial or anti-inflammatory activities in a greater or lesser extent. Phenols and phenolic acids are bioactive phytochemicals consisting of a single substituted phenolic ring. Toxicity of phenols and phenolic acids to microorganisms is due to the site(s) and number of hydroxyl groups present in the phenolic compound (Urs and Dunleavy, 1975). Quinones are characteristically highly reactive, colored compounds with two ketone substitutions in aromatic ring. Flavones, flavonoids and flavonols are phenolic structure with one carbonyl group. They are synthesized by plants in response to microbial infection (Dixon *et al.*, 1983) and are often found effective *in vitro* as antimicrobial substance against a wide array of microorganisms (Bennet and Wallsgrove, 1994). Tannins are polymeric phenolic substances possessing the astringent property. These compounds are soluble in water, alcohol and acetone and give precipitates with proteins (Basri and Fan, 2005). Coumarins are also phenolic and several of them have been reported to have antimicrobial properties.

T reatm ent Name/Test	Concentration (%)				Percent Inhibition * at Different Treatment Concentration (%)								
Plants	Control	10	20	30	40	50	M ean	10	20	30	40	50	M ean
Allium sativum	64.00	49.40	45.70	43.00	32.00	24.70	38.96	22.81 (4.77)	28.59 (5.34)	32.81 (5.72)	50.00 (7.06)	61.40 (7.83)	39.12 (6.25)
Zingiber officinale	65.50	52.40	44.90	41.20	34.60	30.90	40.80	20.00 (4.47)	31.45 (5.60)	37.10 96.09)	47.17 (6.86)	52.82 (7.26)	37.70 (6.14)
Allium cepa	64.80	53.10	48.60	44.10	40.50	36.00	44.46	18.05 (4.24)	25.00 (5.00)	31.94 (5.64)	37.50 (6.12)	41.44 (6.66)	31.38 (5.60)
Mentha spicata	63.00	52.30	49.70	46.20	40.90	36.40	45.10	16.98 (4.10)	21.11 (4.59)	26.70 (5.16)	35.07 (5.92)	42.22 (6.49)	28.41 (5.33)
Curcuma longa	65.00	57.40	54.50	52.50	48.80	4 5 .0 0	51.64	11.70 (3.42)	16.15 (4.01)	19.07 (4.37)	24.92 (4.99)	30.76 (5.55)	20.52 (4.52)
M ean		52.92	48.68	45.40	39.36	34.60		17.90 (4.23)	27.69 (5.26)	29.52 (5.43)	38.93 (6.23)	46.32 (6.80)	

Table 1: Effect of Different Aqueous Plant Extracts on Growth of <i>Fusarium oxysporum</i> f.	. sp. <i>ly</i>	copersici
---	-----------------	-----------

$CD_{0.05}$

Treatments (T)	=	0.01963
Concentration (C)	=	0.01963
T x C	=	0.04391

*Mean of three replications; Figures in parenthesis are transformed values

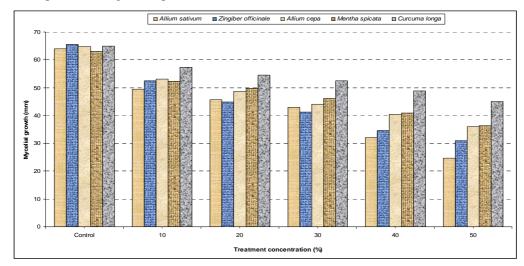


Figure 1: Effect of Different Aqueous Plant Extracts on Mycelial Growth of Fusarium oxysporum f. sp. lycopersici

Remediation of Tomato (Lycopersicon esculentum) Fruit Rot Caused by Fusarium oxysporum f. sp. lycopersici Using Various Plant Extracts

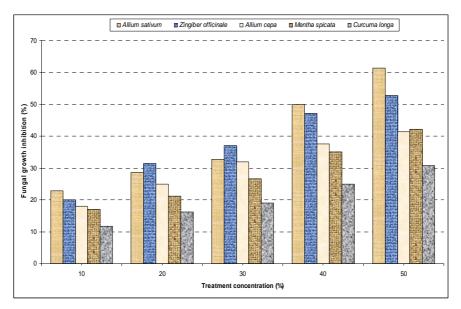


Figure 2: Effect of Different Aqueous Plant Extracts on Inhibition of Mycelial Growth of Fusarium oxysporum f. sp. lycopersici

REFERENCES

- 1. Anwar, A. and Khan, F.U. 2001. Effect of aqueous leaf extractsof medicinal plant on the growth of rhiozspheric fungi of tomato cv. Pusa Ruby *in vitro*. *SKUAST Journal of Research*, **3**: 60 63.
- Arya, A., Chauhan, R. and Arya, C. 1995. Inhibition of growthof 200 pathogenic fungi by garlic extract. *Mycologia*, 67:882 - 885.
- Baris, O., Gulluce, M., Sahin, F., Ozer, H., Kilic, H., Ozkan, H., Sokmen, M. and Ozbek, T. 2006. Biological activities of the essential oil and methanol extract of *Achillea Biebersteinii Afan (Asteraceae). Turkish Journal of Biology* 30: 65-73.
- 4. Basri, D.F. and Fan, S.H. 2005. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. *Indian Journal of Pharmacology* **37**(1): 26-29.
- 5. Bennett, R.N. and Wallsgrove, R.M. 1994. Secondary metabolites in plant defense mechanisms. *Phytotherapy Research* **127**(4): 617-633.
- 6. Bowers, J.H. and Locke. 2000. Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of Fusariumwilt in the green house. *Plant Disease* **3**: 300-305.
- 7. Datar, V.V. 1999. Bioefficacy of plant extracts against *Macrophomona phaseolina* (Tassi) Goid, the incitant to charcoal- rot of sorghum. *Journal of Mycology and Plant Pathology*, **29**: 251-253.
- Dixon, R.A., Dey, P.M. and Lamb, C.J. 1983. Phytoalexins: enzymology and molecular biology. *Advances in Enzymology*55: 1-69.
- Grover, R.K. and Moore, J.D. 1962. Toximetric studies of fungicides against brown rot organisms, *Sclerotia fructicola* and *S. laxa. Phytopathology*52: 876-880.

- 10. Hasabnis, S.N. and D'Souza, T.F. 1988. Use of natural Plant products in the control of the storage rot of Alphanso Mango fruits. *Journal of Maharashtra Agriculture University* **12**(1): 105-106.
- Jacob, C.K. and Sivaprakasam, K. 1994. Evaluation of some plant extracts and antogonsists for the control of preemergence damping-off of brinjal (*Solanum melongena* L.) In: Crop Disease-innovative Techniques and management (Sivaprakaasam, K. ed.). Kalayni Publisher, New Delhi, 289-294 PP.
- 12. Misra, S.B. and Dixit, S.N. 1976. Fungicidal spectrum of the leaf extract of *Allium sativum*. *Indian Phytopathology*, **29**: 448-449.
- Nisa, T., Wani, A. H. and Mir, R. A. 2010. Antimycotic activity of plant extracts on the spore germination of some pathogenic fungi. *Mycopathology* 8(2):65-69.
- Nisa, T., Wani, A.H., Bhat, M.Y., Pala, S.A. and Mir, R.A. 2011. *In vitro* inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. *Journal of Biopesticides* 4(1): 53-56.
- 15. Okigbo, R.N. and Nmeka, I.A. 2005. Control of yam tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. *African Journal of Biotechnology* **4**(8): 804-807.
- 16. Rick, C.M. 1978. The tomato. Scientia Americana 239(2): 76.
- 17. Sahayaraj, K., Namasivayam, S.K.R. and Borgio, J. A. F. 2006. Influeunce of three plant extracts on *Fusarium oxysporum* f. sp. *ciceria* mycelium growth. *Journal of Plant Protection Research*, **46** (4): 335 338.
- 18. Sheik, A. and Agnihotri, J.P. 1972. Antifungal properties of some plant extracts. *Indian Journal of Mycology and Plant Pathology* **2**: 142-146.
- 19. Sindhan, G.S., Hooda, I. and Parashar, R.D. 1999. Evaluation of plant extracts for the control of powdery mildew of pea. *Journal of Mycology and Plant Pathology* **29**: 257-258.
- 20. Sokhi, S.S. and Sohi, H.S. 1982. Assessment of losses in tomato caused by buckeye rot. *IndianPhytopathology* **35**: 675-676.
- 21. Surekha, C., Kumar, K.V.C. and Lakshmipathy, R. 2010. Enhancement of shelf life of tomatoes using herbal extracts. *Journal of Phytology* **2**(1): 13–17.
- 22. Urs, N.V.R.R and Dunleavy, J.M. 1975. Enhancement of the bactericidal activity of a peroxidase system by phenolic compounds (*Xanthomonas phaseoli* var. *sojensis*, soybeans). *Phytopathology* **65**: 686-690