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Remediation of Tomato (*Lycopersicon esculentum*) Fruit Rot Caused by *Phytophthora nicotianae* using Various Plant Extracts

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ABSTRACT

Fungal infections are one of the major causes of post-harvest rots of fresh fruits and vegetables whether in transit or storage. They cause significant economic losses in the commercialization phase, and are rendered unfit for human consumption. There are many reports on the antimicrobial properties of plant extracts containing different classes of phenolic compounds. The Phenolic compounds represent a rich source of preservatives that have been explored for a long time as postharvest alternative control measures to fungicides. The *in vitro* assaying of aqueous and ethanol extracts of five different plants viz. *Allium cepa*, *Allium sativum*, *Curcuma longa*, *Mentha spicata* and *Zingiber officinale* at different concentrations viz., 10, 20, 30, 40 and 50 per cent in comparison with control (without extracts) was carried out in the laboratory for their efficacy against the pathogen *Phytophthora nicotianae* using Poison Food Technique. All tested plant extracts produced some antifungal activities although the rate of inhibition of test fungus varied with the different plant extracts and concentrations. The results revealed that all the tested plant extracts inhibited growth of pathogenic fungus but *Allium sativum* has shown best antifungal activity at all the concentrations both in aqueous and ethanol solvents. It was also observed that the effectiveness of extracts increased with the increase in extract concentration and the maximum inhibition in mycelial growth was found at highest concentration. However, ethanolic plant extracts proved to be more effective than aqueous plant extracts.

***Key words*:** Remediation, Fungi, Plant extracts, Tomato

Highly toxic synthetic chemical warfares have been used to kill the enemy soldiers and people during the world wars. After the World War II, the manufacturers of the toxic synthetic chemicals had shifted their business interest to use them in agriculture as pesticides to kill the pests of various kinds. Hence, these highly toxic synthetic pesticides have huge popularity in terms of sale and usage, which no doubt have big tendency of killing various types of pests attacking and damaging the crops. In the country the "Green Revolution" was launched with a theory of "Chemical Agriculture", which allowed indiscriminate use of toxic synthetic pesticides on crops and post-harvest produce that has crossed all ethical norms of the environmental and human health. Plant diseases due to pathogenic fungi alone cause nearly 20 per cent reductions in the yield of major crops and product damage in much larger extent after harvest. To obtain quick results with less effort, traditional agricultural practices have been replaced by the use of synthetic chemicals for the management of plant pathogens. This has no doubt provided immediate result but with deterioration of environmental quality and human health. In addition to target pathogens, pesticides may also kill various beneficial organisms and their toxic forms can persist in the environment for a longer period [1-2].

The increasing incidences of resistance in pathogens to synthetic chemicals have recently been a source of concern. The adverse effects of the synthetic chemicals on a human health and the environment have contributed in growing interest in alternative control measures. Among various alternatives, natural plant products that are degradable, ecologically friendly and readily available products are catching attention of scientists all over the world. Natural plant products are important sources of new agricultural chemicals [3-4] used to control insect pest [5] and plant diseases [6-9]. According to [10] such products from plants are relatively broad spectrum; bio-efficacious, economical and environmentally safe and can be ideal for use as agrochemicals [11].

The presence and growth of fungi may cause spoilage of food quality and quantity [12]. The growing concern about safety of food has led to development of natural antimicrobials for food preservation [13]. The Plant extracts

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have demonstrated antimicrobial effects as a result of several compounds such as phenolics, flavonoids, allicin, thiosulfonates, betalian, phytoalxins etc. [14-15]. Thus, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action. Therefore, the present study was undertaken to investigate the antifungal activity of aqueous and ethanol extracts of five different plants viz., *Allium cepa*, *Allium sativum*, *Curcuma longa*, *Mentha spicata* and *Zingiber officinale* at different concentrations against pathogen *Phytophthora nicotianae*. Inhibitory effect of methanolic extracts of *Aframomum melegueta*, *Z. officinale* against *Helminthosporium solani*, *Penicillium digitatum*, *Mucorpiriformis* an *A. niger isolated* from the *L. esculentum* at different concentrations ranging from 5-30 per cent [16]. The results revealed that at 15 per cent concentration of each extract, *M. piriformis* and *H. solani* was the most inhibited by the plant extracts, while *A. niger* was least inhibited. However, at 25 per cent concentration, the 4 pathogens were completely inhibited by *Z. officinale* extract. *A. melegueta* extract inhibited completely *H. solani* and *M. piriformis*, while the *P. digitatum* and *A. niger* were 93 and 89 per cent inhibited respectively at 25 per cent concentration of the extract

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 two solvents viz., sterile distilled water and ethanol as per the procedure given by Sindhan *et al*. [17] and Dubey and Dwivedi [18] respectively. Botanicals and their parts used for the preparation of extracts which were obtained from local markets are given below:

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

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.

Ethanol extract

For obtaining the ethanol extract, fresh plant material was collected, washed and dried at room temperature, crushed and suspended in 80% ethanol and filtered after one hour through Whatman filter paper (No. 1). All the filtrates were centrifuged at 5000 rpm for 20 minutes and the supernatants were collected in 100 ml Erlenmeyer flasks. Ethanolic extracts were evaporated to dryness in vacuum rotary evaporator. On cooling their aqueous suspensions were prepared in the ratio 1:1 (w/v).

Determination of antifungal activity of plant extracts on fruit rot pathogens of tomato by Poison Food Technique

The Poison Food Technique (Grover and Moore [19] 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 given below:

$$\% \text{ Mycelial inhibition} = \frac{\text{Mycelial growth (C)} - \text{Mycelial growth (T)}}{\text{Mycelial growth (C)}} \times 100$$

Where;
(C) = Control
(T) = Treatment

RESULTS AND DISCUSSION

Effect of aqueous extract

The results of *in vitro* antifungal activity of aqueous plant extracts are summarized in (Table 1, Fig 1-2). Extracts produced different levels of antifungal activity against *P. nicotianae*. Results indicated that all the extracts significantly reduced the growth of *P. nicotianae*, in comparison with the control. It was observed that on an average, maximum inhibition (42.73%) in the growth of test pathogen was exhibited by *A. sativum* followed by *A. cepa* (39.95%) and *Z. officinale* (32.98%). The lowest inhibition (27.78%) was exhibited by *C. longa* against *P. nicotianae*. However, all test plants significantly inhibited the growth compared to the respective control. The treatment concentrations were found to exert statistically significant differences irrespective of test plants. The growth inhibition significantly increased with increase in treatment concentration such that a minimum average inhibition (20.25%) was recorded at the lowest test concentration 10 per cent and highest average inhibition (51.19%) was recorded at the highest extract concentration of 50 per cent. A significant interaction between test plants and their aqueous treatment concentrations also existed. *A. sativum* at 50 per cent concentration provided maximum growth inhibition (63.06%). *A. cepa* at 50 per cent concentration was the next best test plant extract providing growth

inhibition of (61.27%) followed by *Z. officinale* and *M. spicata* with growth inhibition of 46.55 and 43.04 per cent, respectively. *C. longa* at 10 per cent concentration provided lowest growth inhibition 15.69 per cent.

Table 1 Effect of different aqueous plant extracts on growth of *Phytophthora nicotianae*

Treatment name/test plants	Mycelial growth (mm)*at different treatment concentration (%)							Per cent inhibition* at different concentration (%)					
	Control	10	20	30	40	50	Mean	10	20	30	40	50	Mean
Allium sativum	55.50	41.80	36.70	32.50	27.40	20.50	31.78	24.68 (4.96)	33.87 (5.81)	41.44 (6.43)	50.63 (7.11)	63.06 (7.94)	42.73 (6.53)
Zingiber officinale	52.20	42.60	38.30	34.80	31.30	27.90	34.98	18.39 (4.27)	26.62 (5.15)	33.33 (5.77)	40.03 (6.32)	46.55 (6.82)	32.98 (5.74)
Allium cepa	53.20	41.20	36.90	32.60	28.40	20.60	31.94	22.55 (4.74)	30.63 (5.53)	38.72 (6.22)	46.61 (6.83)	61.27 (7.82)	39.95 (6.32)
Mentha spicata	54.60	43.70	39.10	37.80	33.60	31.10	37.06	19.96 (4.46)	27.60 (5.25)	30.76 (5.54)	38.46 (6.19)	43.04 (6.56)	31.96 (5.65)
Curcuma longa	51.60	43.50	40.30	37.90	34.70	29.90	37.26	15.69 (3.95)	21.89 (4.67)	26.55 (5.14)	32.75 (5.72)	42.05 (6.49)	27.78 (6.48)
Mean		42.56	38.26	35.12	31.08	26.00		20.25 (4.50)	28.12 (5.30)	34.16 (5.84)	41.69 (6.45)	51.19 (7.15)	

$CD_{0.05}$ Treatments (T) = 0.03018 Concentration (C) = 0.03018 T × C = 0.06752

*Mean of three replications; Figures in parenthesis are transformed

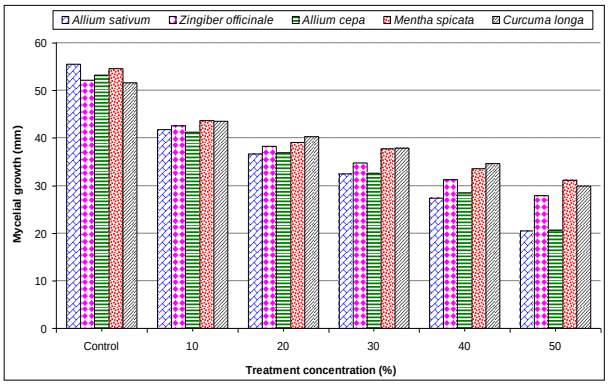

Fig 1 Effect of different aqueous plant extracts on mycelial growth of *Phytophthora nicotianae*

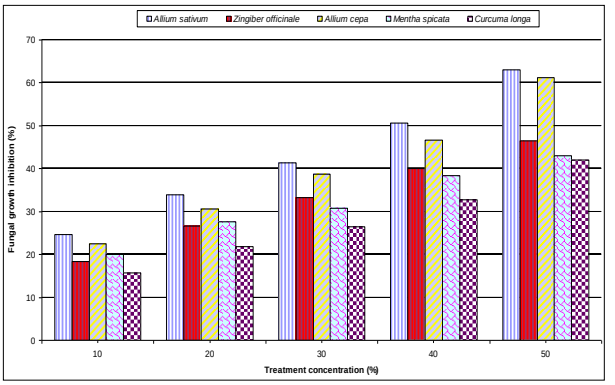

Fig 2 Effect of different aqueous plant extracts on inhibition of mycelial growth of *Phytophthora nicotianae*

Effect of ethanol extract

The ethanolic extracts, on assaying for inhibition in gr owth of *P. nicotianae* (Table 2, Fig 3-4) again indicated the supremacy of *A. sativum* over other plant extracts. On an average, *A. sativum* exhibiting the maximum inhibition (67.30%) was followed by *A. cepa* (55.44%) and *Z. officinale* (52.60%). The ethanolic extract of *C. longa* again exhibited the lowest (45.54%) inhibition of growth of test pathogen. All the treatments, however, significantly inhibited the growth of test pathogen over the respective control. The treatment concentrations were found to exert statistically significant differences, irrespective of test plants. The inhibition in growth significantly increased with increase in treatment concentration. On an average the lowest concentration (10%) exhibited minimum inhibition (38.40%) and highest average inhibition (69.26%) was recorded at the highest treatment concentration of 50 per cent. There also existed a significant interaction between test plants and their ethanolic treatment concentrations. At 50 per cent concentration *A. sativum* provided maximum growth inhibition of (90.72%). *A. sativum* at 40 per cent concentration providing a growth inhibition of 75.27 per cent proved better than the *A. cepa* evaluated at the 50 per cent concentration providing 70.81 per cent growth inhibition of *P. nicotianae*. 10 per cent concentration of *M. spicata* provided growth inhibition of 32.46 per cent followed by *C. longa* 31.55 per cent which were lowest inhibitory among all other test plants.

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 [20]. 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 acid 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 [21]. 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 [22] and are often found effective *in vitro* as antimicrobial substance against a wide array of microorganisms [23]. Tannins are polymeric phenolic substances possessing the astringent property. These compounds are soluble in water, alcohol and acetone and give precipitates with proteins [24]. Coumarins are also phenolic and several of them have been reported to have antimicrobial properties.

Table 1 Table 2 Effect of different ethanolic plant extracts on growth of *Phytophthora nicotianae*

Treatment name/test plants	Mycelial growth (mm)*at different treatment concentration (%)							Per cent inhibition* at different concentration (%)					
	Control	10	20	30	40	50	Mean	10	20	30	40	50	Mean
Allium sativum	55.00	27.90	23.80	19.50	13.60	5.10	17.98	49.27 (7.02)	56.72 (7.53)	64.54 (8.03)	75.27 (8.67)	90.72 (9.52)	67.30 (8.20)
Zingiber officinale	52.00	32.10	27.80	24.20	21.70	17.40	24.64	38.26 (6.18)	46.53 (6.82)	53.46 (7.31)	58.26 (7.63)	66.53 (8.15)	52.60 (7.25)
Allium cepa	51.40	30.60	26.60	23.20	19.10	15.00	22.90	40.46 (6.35)	48.24 (6.94)	54.86 (7.40)	62.84 (7.92)	70.81 (8.41)	55.44 (7.44)
Mentha spicata	53.90	36.40	33.00	30.00	24.00	21.00	28.88	32.46 (5.69)	38.77 (6.22)	44.34 (6.65)	55.47 (7.44)	61.03 (7.81)	46.41 (6.81)
Curcuma longa	52.60	36.00	32.00	28.00	24.70	22.50	28.64	31.55 (5.61)	39.16 (6.25)	46.76 (6.83)	53.04 (7.28)	57.22 (7.56)	45.54 (6.82)
Mean		32.60	28.64	24.98	20.62	16.20		38.40 (6.19)	45.88 (6.77)	52.79 (7.26)	60.97 (7.80)	69.26 (8.32)	

$CD_{0.05}$ Treatments (T) = 0.347 Concentration (C) = 0.347 T × C = 0.077

*Mean of three replications; Figures in parenthesis are transformed

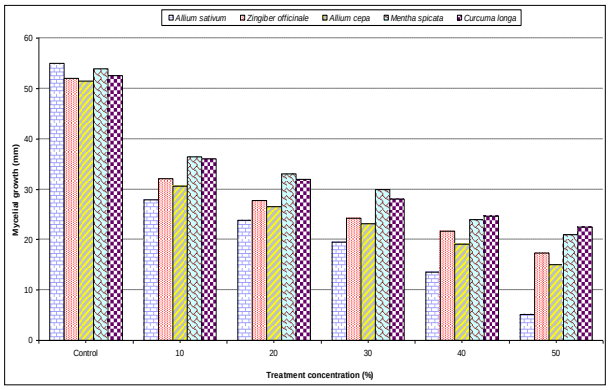

Fig 3 Effect of different ethanolic plant extracts on mycelial growth of *Phytophthora nicotianae*

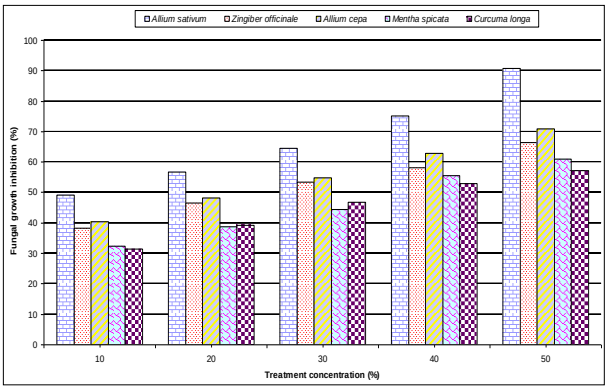

Fig 4 Effect of different ethanolic plant extracts on inhibition of mycelial growth of *Phytophthora nicotianae*

It was observed from the results that both the aqueous and ethanolic extracts were effective against all test fungi but ethanolic extracts showed better results as compared to aqueous as being organic, dissolves more organic compounds resulting in the release of greater amount of active antimicrobial components. These findings indicate usefulness of the plant extracts to keep the diseases away. Similar results with plant extracts have also been reported by some workers. Neem leaf diffuse and neem leaf powder completely inhibitory to *Phytophthora infestans* [25]. 50.00% inhibition of *Phytophthora nicotianae* and *Phytophthora citricola* with botanical *Pinus radiata* as recorded by [26], [27] recorded that 1:3 concentration of Allamanda leaf extract was completely inhibitory to *Phytophthora capsica*, while [28] recorded 81% inhibition of *P. capsici* by botanical coir extract after 8 days at 0.24 mg/ml concentration and [29] recorded 98.4 to 99.9% inhibition of *Phytophthora nicotianae* in greenhouse by 10% aqueous emulsion of clove oil and cinamom oil. [30] noted inhibitory effect of olive extract (*Olea europaea* L.) against *Phytophthora spp*. and [31-32] also tested botanicals against *Pythium aphinidermatum*, while [33] noted total inhibitory effect of spider lilly (*Crinum asiaticum*) against *P. aphinidermatum* and [34] reported 60% inhibition of *P. aphinidermatum* with neem formulations. Inhibitory effect of *Allium sativum* and *Azadirachta indica* against *P. aphinidermatum* as reported by [35].

CONCLUSION

All the aqueous and ethanolic plant extracts proved to be effective against the test pathogenic fungi. However, ethanolic plant extracts proved to be more effective than aqueous plant extracts. Among various plant extracts *A. sativum* was most effective.

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