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Study on *in-vitro* efficacy of botanicals and chemicals against *Alternaria solani* associated with post-harvest rot of tomato (*Lycopersicon esculentum* Mill.)

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Abstract

Tomato (*Lycopersicon esculentum* Mill.) is one of the major vegetable fruit crop in the state of Maharashtra and widely grown in Marathwada region throughout year. It is known to be affected by several fungal diseases. Based on symptomatology, microscopic observations and pathogenicity test, the test pathogens were identified as *Alternaria solani* and its further identity was confirmed. The pathogen (*Alternaria solani*) was isolated successfully from the naturally infected fruits of tomato. Culture was purified and maintained for further studies. In *in vitro* study, all tested botanicals/plant extracts were found effective in inhibition of mycelial growth of *A. solani*. Least mycelial growth (00.67 mm) was recorded with maximum mycelial growth inhibition with Garlic extract (99.25%) which was at par with treatment of Nilgiri oil (97.77%) and followed by Ginger rhizome extract (79.63%), Tulsi (70.74%), and Onion bulb extract (70.00%). While least mycelial inhibition was recorded with Turmeric rhizome extract (64.82%) and Neem leaf extract (57.04%). All the tested chemicals were found fungistatic against *A. solani*. Calcium chloride and Boric acid at both concentration 1 & 2% were found most effective in inhibiting the mycelial growth of *Alternaria solani*, while least inhibition of these fungi was observed with Potassium chloride and Sodium bicarbonate.

Keywords: Plant extract, chemicals, fruit rot, tomato, *Alternaria solani*

Introduction

Tomato (*Lycopersicon esculentum* Mill.) belongs to family solanaceae is a weak herbaceous plant capable of perennial growth, but normally cultivated as an annual. It is an important fruit vegetable and ranks next to potato in world acreage and is first amongst processing crops. It is a warm-season vegetable crop grown extensively in cool season. Because of its wider adaptability and versatility, tomato is grown throughout the world. India is second largest producer of tomato next to china. In India, the area estimated during 2017-18 under tomato cultivation was 801000 hectare with production of 22337 thousand metric tonnes having productivity of 24.4MT per hectare. In Maharashtra estimated area under tomato was 43.64 thousand hectare during the year 2016-17 with production of 957.17thousand metric tonnes and the average productivity was 22 metric tonnes per ha (Anonymous, 2017) [2]. It is a rich source of vitamin A and C; it also contains minerals like Mg, Ca, P, Fe, Na, K, Cu, S and Cl. It is used in diverse ways, including raw in salads and processed into ketchup or tomato soup. Bacteria, fungi, viruses, phytoplasma, viroids, nematodes, insects and parasitic phanerogams are the major causes of parasitic diseases. Fruit rot is one of the major limiting factor in tomato cultivation causing rotting of tomatoes by microorganisms between harvest and consumption which ultimately make tomato fruits unfit for consumers. Tomato is a very perishable vegetable with short shelf life and due to their low pH, higher moisture and nutrient composition make them highly susceptible to fungal diseases causing fruit rots. Improper harvesting, handling, packaging and transportation may result in bruises, decay and development of microorganisms. Change in physiological state of fruit and storage condition make favorable environment for spoilage of fruit. Fajola (1979) [8] reported 25% loss at harvest and 34% loss of the remaining product in transit, storage and market due to post-harvest fruit rot diseases of tomato in five states of Nigeria Chinoko and Nagvi (1989) [6] isolated 243 fungi associated with post-harvest rot of tomato from eight marketing sites in Logos and Oyo states. Akthar *et al.* (1994) [6] reported the susceptibility of tomato to post-harvest disease caused to fungal pathogens during prolonged storage conditions. Prevalence of fungal fruit rot of tomato occurred throughout year causing more or less damage, but maximum losses occurred during warm and humid condition (Chavan, 2012) [5].

The disease appearing in field and disease encountered after harvest are complementary to each other and need concurrent investigation in order to provide adequate and scientific protection not only to growing plants in the field but also to plant produce after harvest during storage and transit. Since not much information is available regarding the diseases of tomato after harvest in Marathwada region of Maharashtra. It is felt necessary to undertake the investigation on postharvest fungal diseases of tomato fruit. Present investigation was conducted to study *in-vitro* efficacy of botanicals and chemicals against *Alternaria solani* associated with post-harvest rot of tomato.

Material and Methods

Present research work entitled “Study on *in-vitro* efficacy of botanicals and chemicals against *Alternaria solani* associated with post-harvest rot of tomato (*Lycopersicon esculentum* Mill.)” was conducted at the Department of Plant Pathology college of Agriculture, Badnapur and Agriculture Research Station, Badnapur of VNMKV, Parbhani during the year 2017-18. The materials used and methods adopted during the course of investigation are described here.

General Laboratory Procedure

Throughout the investigation, Borosil made Petri plates, conical flasks and test tubes were used. The cleaned dried glass wares were sterilized in hot air oven at 180°C for one hour. The media and distilled water were sterilized in autoclave at 15lb pressure for 15 minutes.

Collection

Diseased (rotten) tomato fruits were collected from different local marketing sites of Dist. Jalna. Collections of rotten samples were done randomly from each place. Randomly selected diseased fruits from each place were brought to the laboratory of department of Plant Pathology in clean polythene bags for further study.

Isolation of *Alternaria solani*

Numbers of isolations were made from diseased fruits separately on potato dextrose agar (PDA) medium by usual isolation method. The infected samples were cleaned with sterile water so as to remove extraneous material. After air drying, small pieces of infected portion along with healthy portion were taken. These pieces were surface sterilized with 0.1% mercuric chloride for 1 minute and then washed several times with sterilized water to remove the traces of the disinfectant if any. The pieces were dried on flame and were plated under aseptic condition on agar medium previously sterilized at 15 lb pressure for 15 minutes. The Petri plates were incubated at room temperature (27± 2°C) until proper growth of fungi was obtained. Growths of fungi were obtained within 3 to 5 days in all Petri plates. Bits of small mycelial growth from the typical colonies were transferred on slants of PDA under aseptic condition. The isolates were maintained separately in pure state on PDA slants for further studies.

Pathogenicity of *Alternaria solani*

Fresh and healthy tomatoes of uniform size at colour breaking stage were surface sterilized with 0.1% mercuric chloride for 1 minute and rinsing them with three successive changes of

Distilled water. Fruits were pin pricked to a depth of 2-3 mm. The freshly grown bits of respective pathogens were placed over the injured portion. A small piece of moist absorbent cotton was covered over the inoculated fruits to avoid the drying before establishment of host pathogen contact. Four tomato fruits were inoculated with each of the isolates replicated thrice. Another set of four fruits with wounds, but not inoculated, served as control. Inoculated and controls fruits were kept inside the moist beljar, where humidity was maintained to near saturation point by means of frequent sprays of sterile water. After 72 hrs of inoculation, fruits were observed for symptom developments. Reisolation from artificially infected tomatoes was undertaken. The fungal cultures obtained on PDA by reisolation were compared with the original culture obtained from naturally infected tomato fruits and identified using cultural and morphological features.

In-vitro evaluation of phyto extracts and chemicals against *Alternaria solani* associated with tomato rot

The present investigation was carried out to evaluate different plant extracts *viz.*, ginger, turmeric, neem, garlic, nilgiri, onion and tulsi for the possible presence of fungitoxic properties against *Alternaria solani* associated with tomato rot. The efficacy was tested through by Poisoned Food Technique. The plant extracts were prepared by adopting aqueous plant extract solution. The standard aqueous extracts of plant materials were obtained by grinding the appropriate washed plant materials in mortar and pestle in presence of equal amount of sterile distilled water. Prepared plant extracts were filtered through three folds of muslin cloth. The plant extracts along with requisite concentration used are given in Table 1.

The effect of boric acid, calcium chloride, sodium bicarbonate and potassium chloride were tested *in-vitro* separately at 1% and 2% concentrations on mycelial growth of major fungi associated with tomato rot. The chemicals along with requisite concentration used are given in Table 2.

Potato dextrose agar medium was prepared and distributed at the rates, 100 ml in 250 ml conical flask and autoclaved at 15 lb for 15 minutes. Before solidification of media different plant extracts with desired concentration were incorporated aseptically in flasks. These flasks were shaken thoroughly and poured in Petri plates at the rate of 20 ml /plate. Three plates for each treatment were maintained to serve as three replication. One set of three plates was poured without plant extracts to serve as control. The 5 mm mycelial disc of test pathogen selected from peripheral growth of the plate by cork borer were used for inoculating the plates by keeping one disc per plate in the centre. The inoculated plates were kept in the inverted position. Plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogen on medium was recorded and per cent inhibition over control was calculated by the following formula of Horsfall (1956)^[9].

$$X = \left[\frac{Y - Z}{Y} \times 100 \right]$$

Where, X = Per cent inhibition,

Y = Growth of fungus in control (mm)

Z = Growth of fungus in treatment (mm)

Table 1: List of plant extracts used against *Alternaria solani* associated with tomato rot

Sr. No.	Botanical name	Common name	Family	Plant Part used	Conc. (%)
1.	<i>Zingiber officinale</i>	Ginger	Zingiberaceae	Rhizome	10%
2.	<i>Curcuma longa</i>	Turmeric	Zingiberaceae	Rhizome	10%
3.	<i>Azadirachta Indica</i>	Neem	Meliaceae	Leaves	10%
4.	<i>Allium sativum</i> L.	Garlic	Liliaceae	Clove	10%
5.	<i>Eucalyptus globules</i>	Nilgiri	Myrtaceae	Oil	10%
6.	<i>Allium cepa</i>	Onion	Liliaceae	Bulb	10%
7.	<i>Ocimum sanctum</i>	Tulsi	Liliaceae	Leaves	10%
8.	Control				

Table 2: List of chemicals used against *Alternaria solani* associated with tomato rot

Sr. No.	Name of chemicals	Concentration (%)
1.	Boric acid	1% and 2%
2.	Calcium chloride	1% and 2%
3.	Potassium chloride	1% and 2%
4.	Sodium bicarbonate	1% and 2%
5.	Control	

Table 3: *In-vitro* efficacy of plant extracts against *Alternaria solani* with tomato rot

Tr. No.	Treatment	Colony Dia.* (mm)	Per cent Inhibition
T1	Ginger (<i>Zingiber officinale</i>)	18.33	79.63
T2	Turmeric (<i>Curcuma longa</i>)	31.66	64.82
T3	Neem (<i>Azadirachta Indica</i>)	38.66	57.04
T4	Garlic (<i>Allium sativum</i> L.)	00.67	99.25
T5	Nilgiri (<i>Eucalyptus globules</i>)	02.00	97.77
T6	Onion (<i>Allium cepa</i>)	27.00	70.00
T7	Tulsi (<i>Ocimum sanctum</i>)	26.33	70.74
T8	Control	90.00	00.00
	SE±	00.83	
	CD @ 1%	03.30	

Results and Discussion

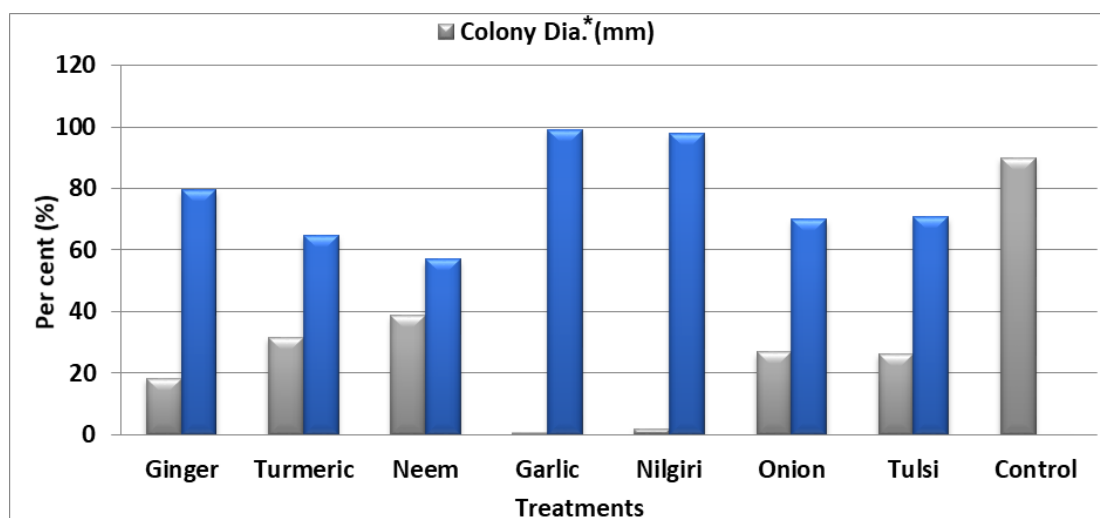
In-vitro efficacy of plant extracts against *Alternaria solani* associated with tomato rot

Efficacy of seven plant extracts were tested using Poisoned Food Technique (PFT) for inhibition of mycelial growth of *A. solani*. Evaluation of different plant extracts for their fungitoxic properties against *Alternaria solani* showed significant inhibition of growth of test fungi *in-vitro* over control. Data (Table 3, PLATE I & Fig.1) revealed that mycelial growth of *Alternaria solani* was recorded from 00.67mm to 38.66 mm as against 90.00 mm in untreated control (Table 3). However, significantly least mycelial growth (00.67 mm) was recorded with maximum mycelial growth inhibition with Garlic extract (99.25%) which was at par with treatment of Nilgiri oil (97.77%) and followed by Ginger rhizome extract (79.63%), Tulsi (70.74%), and Onion bulb extract (70.00%). While least mycelial inhibition was recorded with Turmeric rhizome extract (64.82%) and Neem leaf extract (57.04%).

The results of present investigation resembling the findings of earlier workers *viz.*, Datar (1992) [7] who reported the inhibitory effect of garlic bulb extract on the mycelial growth of *A. tenuis* causal organism of brinjal leaf spot. Prasad and Naik (2003) [11] obtained 90.7 per cent inhibition of *A. solani* with garlic bulb extract. Present findings are supported by the work of Salvi (2007) [12].

**Plate 1:** *Alternaria solani*

In-vitro efficacy of different plant extracts at 10% concentration against *Alternaria solani* associated with tomato rot T₁ Ginger, T₂ Turmeric T₃ Neem, T₄ Garlic, T₅ Nilgiri oil, T₆ Onion, T₇ Tulsi and T₈ Control.

**Fig 1:** *In-vitro* effect of different plant extracts against *Alternaria solani*.

***In-vitro* efficacy of chemicals against *Alternaria solani* associated with tomato rot**

Efficacy of four chemicals *viz.*, Boric acid, Calcium chloride, Potassium chloride and sodium bicarbonate were tested using Poisoned Food Technique (PFT) for inhibition of mycelial growth of *Alternaria solani*. Each chemical at concentrations of 1.0% and 2.0% were tested separately for inhibition of mycelial growth. Results Data (Table 4, PLATE II & Fig.2) indicated that all the chemicals tested (@ 1.0% and 2% each) significantly inhibited mycelial growth of the test pathogen, over untreated control. [PLATE II].

At 1 per cent concentration of chemicals, radial mycelial growth *Alternaria solani* was recorded from 05.50 mm to 10.75 mm as against 89.00 mm in untreated control. However, significantly least mycelial growth (05.50 mm) was observed by recording maximum mycelial growth inhibition of 93.82% with Calcium chloride which was at par with treatment of Boric acid (90.16%), while least mycelial inhibition was recorded with Potassium chloride (88.48%)

and Sodium bicarbonate (87.92%).

Tested chemicals at 2 per cent concentration recorded radial mycelial growth *Alternaria solani* from 2.00 mm to 9.25 mm as against 89.00 mm in untreated control. However, significantly least mycelial growth (2.00 mm) was observed by recording maximum inhibition of (97.75%) with Calcium chloride followed by Boric acid (95.22%), while least mycelial inhibition was recorded with Potassium chloride (91.01%) and Sodium bicarbonate (89.60%).

The results of present investigation are in line with the findings of earlier workers. Patel *et al.* (2005) [10] proved the effectiveness of boric acid against *A. lunata* inciting fruit rot of tomato. Ashour (2009) [3] tested five fungicides, i.e. consento, flent, score, sereno and tridex 8% as well as five antioxidants, i.e. bion, calcium chloride, lithium sulphate, potassium mono- hydrogen-phosphate and salicylic acid and observed significant reduction in the linear growth of *Alternaria solani*, the causal agent of tomato early blight, compared with check treatment.

Table 4: *In-vitro* efficacy of chemicals against *Alternaria solani* associated with tomato rot

Tr. No.	Treatment	Colony Dia.*(mm)	Per cent Inhibition	Colony Dia.*(mm)	Per cent Inhibition
		1%		2%	
T1	Boric acid	08.75	90.16	4.25	95.22
T2	Calcium Chloride	05.50	93.82	2.00	97.75
T3	Potassium Chloride	10.25	88.48	8.00	91.01
T4	Sodium Bicarbonate	10.75	87.92	9.25	89.60
T5	Control	89.00	00.00	89.00	00.00
	SE±	00.82		00.88	
	CD @ 1%	03.35		03.59	

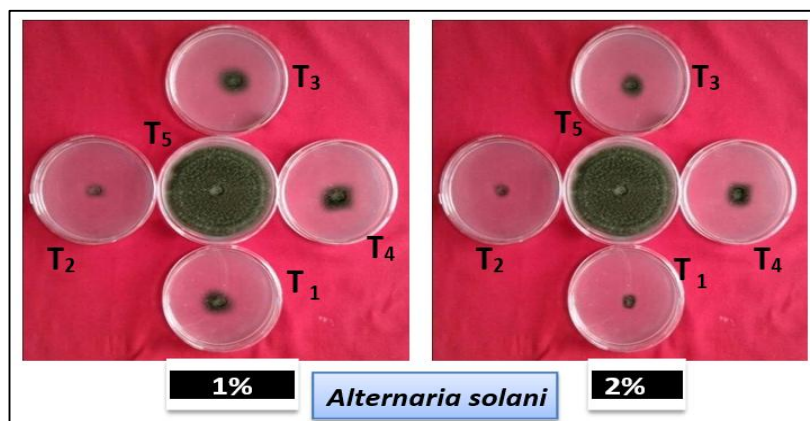


Plate 2: Efficacy of different chemicals against post-harvest fungal diseases of tomato

In-vitro efficacy of different chemicals against post-harvest fungal diseases of tomato. T₁ Boric acid, T₂ Calcium chloride,

T₃ Potassium Chloride, T₄ Sodium bicarbonate and T₅ Control.

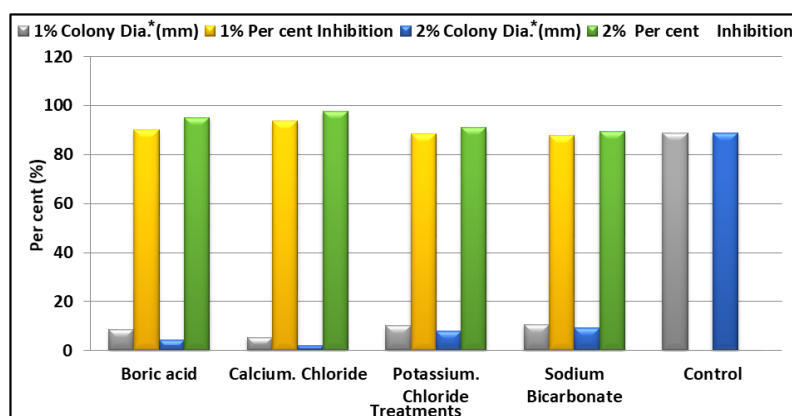


Fig 2: *In-vitro* effect of different chemicals at 1 and 2% concentration against *Alternaria solani*.

Conclusion

Thus, from the results obtained on various aspects during present investigation on *in-vitro* efficacy of botanicals and chemicals against *Alternaria solani* associated with post-harvest rot of tomato, it could be concluded that tomato fruits after harvest were infected by fungal diseases and produced various kinds of rots.

1. Aqueous extract of botanicals viz., Garlic, Turmeric, Tulsi, Ginger, Onion bulb, Neem and Nilgiri oil were found fungistatic against *Alternaria solani*. These botanicals further needed to be tested for economical and eco-friendly management of *Alternaria solani*.
2. All the four chemicals tested *in-vitro* were found to inhibit mycelial growth of *Alternaria solani*. However, CaCl_2 and Boric acid, were highly effective as compared to all other treatments.

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