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Bhalerao JB
M.Sc. Student, Department of Plant Pathology, College of Agriculture, Badnapur, VNMKV, Parbhani, Maharashtra, India

Chavan RA
Associate Professor, College of Agriculture, Osmanabad, VNMKV, Parbhani, Maharashtra, India

Dharbale BB
M.Sc. Student, Department of Plant Pathology, College of Agriculture, Badnapur, VNMKV, Parbhani, Maharashtra, India

Kamble AA
M.Sc. Student, Department of Plant Pathology, College of Agriculture, Badnapur, VNMKV, Parbhani, Maharashtra, India

Bhalerao PB
M.Sc. Student, Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani, Maharashtra, India

Corresponding Author:
Dharbale BB
M.Sc. Student, Department of Plant Pathology, College of Agriculture, Badnapur, VNMKV, Parbhani, Maharashtra, India

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

Bhalerao JB, Chavan RA, Dharbale BB, Kamble AA and Bhalerao PB

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 rotten with typical soft and watery tomato fruit wth coarse grey colour growth yielded the growth of *Rhizopus stolonifer* on artificial media and proved pathogenic in causing fruit rot of tomato in artificial inoculation. 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 *R. stolonife*r. least mycelial growth was recorded with maximum inhibition of 88.15% with treatment of Nilgiri oil which was at par with treatment of Garlic clove extract (84.07%) followed by Turmeric rhizome extract (81.11%). While least mycelial inhibition was recorded with Onion bulb extract (66.30%) and Tulsi leaf extract (64.07%), Neem leaf extract (54.07%) and Ginger rhizome extract (52.96%). All the tested chemicals were found fungistatic against *R. stolonifer*. Calcium chloride and Boric acid at both concentration 1 & 2% were found most effective in inhibiting the mycelial growth of *Rhizopus stolonifer*, while least inhibition of these fungi was observed with Potassium chloride and Sodium bicarbonate.

Keywords: Plant extract, chemicals, fruit rot, tomato, *Rhizopus stolonifer*

Introduction
Tomato (*Lycopersicon esculentum* Mill.) belongs to family solanaceae. 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].

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) [5] 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. Akthar *et al.* (1994) [1] reported the susceptibility of tomato to post harvest disease caused to fungal pathogens during prolonged storage conditions. 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 were conducted to study the *in-vitro* efficacy of botanicals and chemicals against *Rhizopus stolonifer* associated with post harvest rot of tomato.

Materials and Methods

Present research work entitled "Study on *in-vitro* efficacy of botanicals and chemicals against *Rhizopus stolonifer* 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.

Collection of tomato fruits affected with *Rhizopus* rot

Randomly rootten fruits showing showing the symptoms of soft and watery surface of tomato fruit with coarse, gray, hairy mycelial growth with mass of black sporangia at their tips were selected for isolation of *Rhizopus stolonifer*.

Isolation of *Rhizopus stolonifer*

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± 2^0c) 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 *Rhizopus stolonifer*.

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 phytoextracts and chemicals against *Rhizopus stolonifer*

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 *Rhizopus stolonifer* 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) [6].

$$X = \left[\frac{Y - Z}{Y} x100 \right]$$

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 *Rhizopus stolonifer* associated with tomato rot

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

Table 2: List of chemicals used against *Rhizopus stolonifer* 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	

Results and Discussion

In-vitro efficacy of plant extracts against *Rhizopus stolonifer* associated with tomato rot

Seven plant extracts *viz*., Ginger (Rhizome), Turmeric (Rhizome), Neem (Leaf extract), Garlic (clove), Nilgiri (oil), Onion (bulb extract) and Tulsi (Leaf) were evaluated *in-vitro* (each @ 10%) against *Rhizopus stolonifer*. By using Poisoned Food Technique (PFT) for inhibition of mycelial growth of *Rhizopus stolonifer*. Evaluation of different plant extracts for their fungitoxic properties against *Rhizopus stolonifer* showed significant inhibition of growth of test fungi *in- vitro* over control. Data (Table 3, PLATE I & Fig.1) revealed that radial mycelial growth of *Rhizopus stolonifer* was recorded from 10.66 mm to 42.33 mm as against 90.00 mm in untreated control. However, significantly least mycelial growth was recorded with maximum inhibition of 88.15% with treatment of Nilgiri oil which was at par with treatment of Garlic clove extract (84.07%) followed by Turmeric rhizome extract (81.11%). While least mycelial inhibition was recorded with Onion bulb extract (66.30%) and Tulsi leaf extract (64.07%), Neem leaf extract (54.07%) and Ginger rhizome extract (52.96%).

The results of present investigation resembling the findings of earlier workers, Un-Nisa *et al*. (2010) [9] studied the efficacy of extracts obtained from mint leaves, onion bulbs and garlic on the spore germination of *Rhizopus stolonifer* and *Alternaria alternata* higher conc. found highly effective followed by lower conc. The results further indicated that the extract of *Allium sativum* was highly effective as compared to *Allium cepa* and *Mentha arvensis*. Joshi (1985) [7] studied the effect of different plant extracts on pathogens infecting banana fruits and he concluded that application of turmeric rhizome extract (10%) found most effective in inhibiting the growth of several pathogens *viz*., *Fusarium spp*., *Botryodiplodia theobromae*, *Colletotrichum musae*, *Penicillium spp*., *Rhizopus spp.* and *Aspergillus spp.*

Table 3: *In-vitro* efficacy of plant extracts against *Rhizopus stolonifer*

Tr. No.	Treatment	Colony Dia.*(mm)	Per cent Inhibition
T1	Ginger *(Zingiber officinale)*	42.33	52.96
T2	Turmeric *(Curcuma longa)*	17.00	81.11
T3	Neem (*Azadirachta Indica*)	41.33	54.07
T4	Garlic (*Allium sativum* L.)	14.33	84.07
T5	Nilgiri (*Eucalyptus globules*)	10.66	88.15
T6	Onion (*Allium cepa*)	30.33	66.30
T7	Tulsi (*Ocimum sanctum*)	32.33	64.07
T8	Control	90.00	00.00
	SE±	00.97	
	CD @ 1%	03.88	

Plate 1: *Rhizopus stolonifer*

In-vitro efficacy of different plant extracts at 10% concentration against *Alternaria solani* associated with tomato rot

T_1 Ginger, T_2 Turmeric T_3 Neem, T_4 Garlic, T_5 Nilgiri oil, T_6 Onion, T_7 Tulsi and T_8Control.

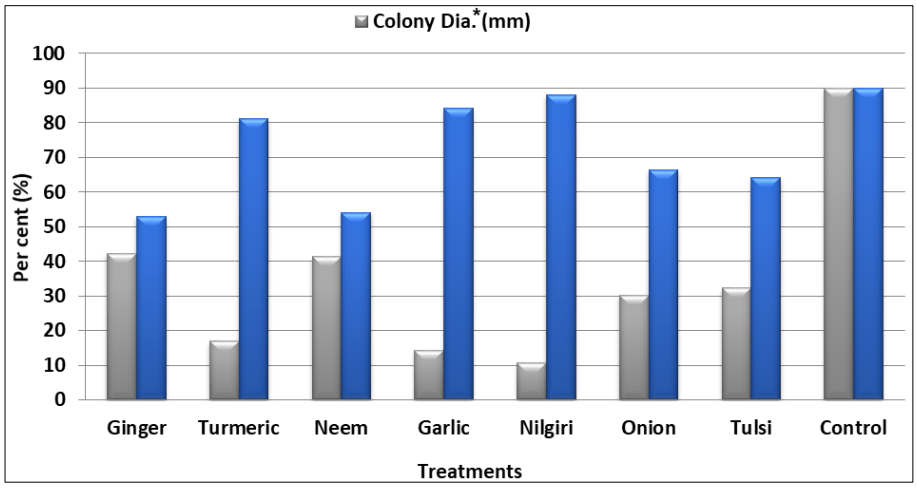

Fig 1: *In-vitro* effect of different plant extracts against *Rhizopus stolonifer*.

In-vitro efficacy of chemical against *Rhizopus stolonifer*

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 *Rhizopus stolonifer*. Each chemical at various concentrations of 1.0% and 2.0% were tested separately for inhibition of mycelial growth. Results obtained on mycelial growth inhibition of *Rhizopus stolonifer* with tested

Chemicals are presented in the Table 4 and depicted in the Fig. 2 and PLATE 2 (a). Results indicated that all the chemicals tested (@ 1.0% and 2% each) significantly inhibited mycelial growth of the test pathogen, over untreated control.

At 1 per cent concentration, radial mycelial growth *Rhizopus stolonifer* was recorded from 13.25 mm to 31.50 mm as against 89.00 mm in untreated control. However, significantly least mycelial growth (13.25 mm) was observed by recording maximum inhibition of 85.11% with Calcium chloride while least mycelial inhibition was recorded with Boric acid (69.10%), Sodium bicarbonate (65.44%) and Potassium chloride (64.60%).

At 2 per cent concentration of chemicals, radial mycelial growth *Rhizopus stolonifer* was recorded from 08.50 mm to 26.75 mm as against 89.00 mm in untreated control. However, significantly least mycelial growth (08.50 mm) was observed by recording maximum inhibition of 90.44% with Calcium chloride while least mycelial inhibition was recorded with Boric acid (79.49%) Potassium chloride (71.91%) and Sodium bicarbonate (69.94%).

The results of present investigation resembling the findings of earlier workers, Nadia *et al*. (2014) [8] reported that potassium sorbate gave complete mycelial growth inhibition of *R. stolonifer* (90.00%) on PDA media followed by calcium chloride (80.99%). Further they reported that potassium sorbate and sodium benzoate found superior in reducing the fruit decay in tomato.

Table 4: *In-vitro* efficacy of chemicals against *Rhizopus stolonifer*

Tr. No.	Treatment	Colony Dia.*(mm)	Per cent Inhibition	Colony Dia.*(mm)	Per cent Inhibition
		1%		2%	
T1	Boric acid	27.50	69.10	18.25	79.49
T2	Calcium Chloride	13.25	85.11	08.50	90.44
T3	Potassium Chloride	31.50	64.60	25.00	71.91
T4	Sodium Bicarbonate	30.75	65.44	26.75	69.94
T5	Control	89.00	00.00	89.00	00.00
	SE±	01.12		01.13	
	CD @ 1%	04.53		04.59	

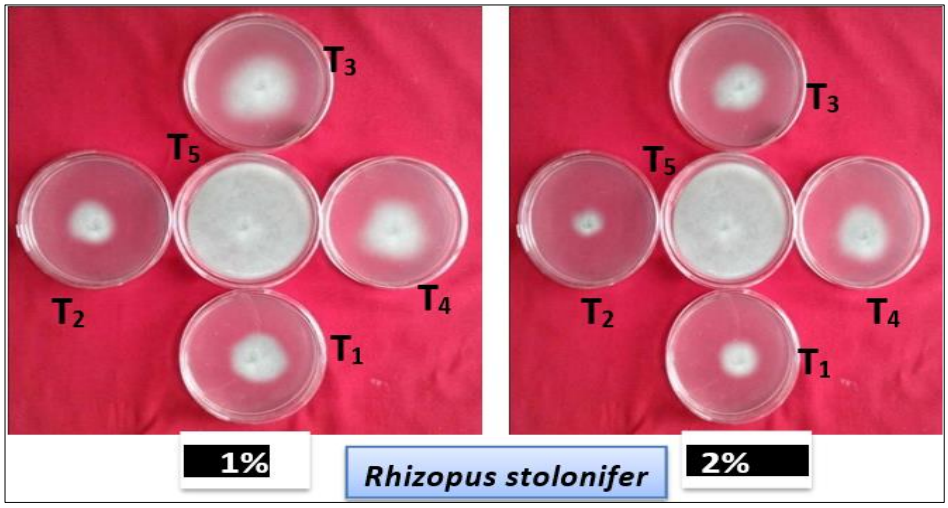

Plate 2: *Rhizopus stolinifer*

In-vitro efficacy of different chemicals against post harvest fungal diseases of tomato.

T_1Boric acid, T_2 Calcium chloride, T_3 Potassium Chloride, T_4 Sodium bicarbonate and T_5 Control.

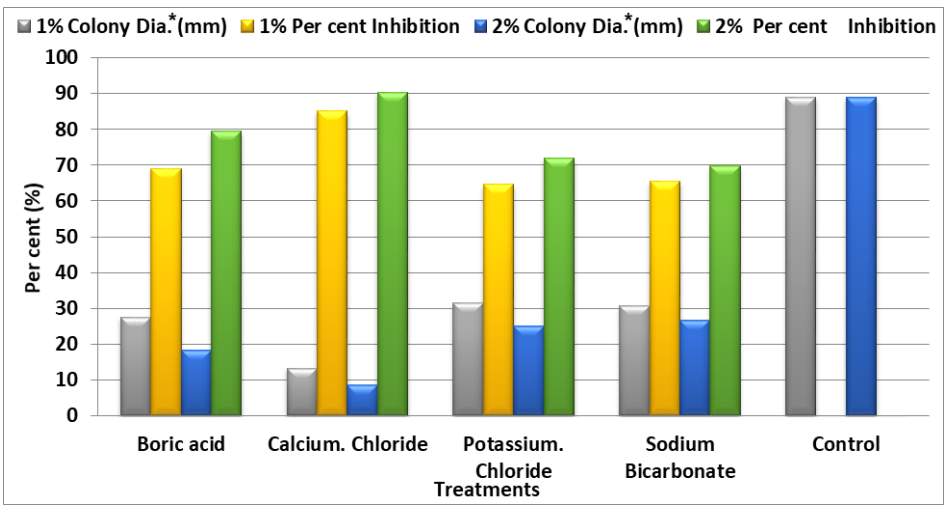

Fig 2: *In-vitro* effect of different chemicals at 1and 2% concentration against *Rhizopus stolonifer*.

Conclusion

Thus, from the results obtained on various aspects during present investigation on *in-vitro* efficacy of botanicals and chemicals against *Rhizopus stolonifer* 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 *Rhizopus stolonifer*. These botanicals further needed to be tested for economical and eco-friendly management of *Rhizopus stolonifer*.

2 All the four chemicals tested *in-vitro* were found to inhibit mycelial growth of *Rhizopus stolonifer* However, $Cacl_2$ and Boric acid, were highly effective as compared to all other treatments.

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