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Studies on *in-vitro* efficacy of chemicals against major fungi associated with post-harvest rot of tomato

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Abstract
Present research work entitled “Studies on *In-vitro* efficacy of chemicals against major fungi associated with post-harvest rot of tomato” was conducted at the Department of Plant Pathology, college of Agriculture, Badnapur and Agriculture Research Station, Badnapur, Dist- Jalna (VNMKV, Parbhani), M.S. during the year 2017-18. The experiment was laid out in Completely Randomised Design-CRD, with three replications and five treatments. The present investigation was carried out to evaluate effect of different chemical like 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. Calcium chloride and Boric acid at both concentration 1 & 2% were found most effective in inhibit the mycelial growth of *Alternaria solani, Aspergillus niger* and *Rhizopus stolonifer,* while least inhibition of these fungi was observed with Potassium chloride and Sodium bicarbonate *Colletotrichum gloeosporioides* gave least growth in treatment of Potassium chloride and Boric acid at 1% while at 2% Calcium chloride inhibited the maximum growth of *C. gloeosporioides* followed by Potassium chloride, while the least average mycelial inhibition was recorded with Sodium bicarbonate and Boric acid 87.64%.

Keywords: *In Vitro,* efficacy, chemicals, fungi, post-harvest loss, tomato

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 originated from tropical America (Bose *et al*., 1986) [3]. Bacteria, fungi, viruses, phytoplasma, viroids, nematodes, insects and parasitic phanerogams are the major causes of parasitic diseases. Similarly, non-parasitic diseases are caused due to extremes light, heat, soil moisture and pH and by nutritional imbalances. Damage caused by herbicides, pesticides, lightning and genetic disorders are also non parasitic diseases (Jones *et al*., 1997) [7]. 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) [7] 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) [5] isolated 243 fungi associated with post-harvest rot of tomato from eight marketing sites in Logos and Oyo states. Akthar *et al.* (1994) [1] 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) [4]. 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 post-harvest fungal diseases of tomato fruit at various markets in the Marathwada region with the following objectives.

1. To isolate and identify fungal pathogens associated with post-harvest rot of tomato.
2. To study *in-vitro* efficacy of chemicals against major fungi associated with post-harvest rot of tomato.

Materials and methods

Present research work entitled "Studies on *In-vitro* efficacy of chemicals against major fungi associated with post-harvest rot of tomato" 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 in this chapter.

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 1800C for one hour. The media and distilled water were sterilized in autoclave at 15lb pressure for 15 minutes.
Identification of fungi associated with tomato rots.

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.

Table 1: List of chemicals used against major fungi 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	

3.2. Statistical analysis

Statistical analysis was carried out as per procedure by Panse and Sukhatme (1967). Data on percentage were transformed in to arc sine values and analysis was done in CRD, also the statistical analysis for multiple, linear regression and correlation were done on the basis of average weekly date of per cent disease intensity and average weekly metrological data using MS office.

Result and discussion

In vitro* efficacy of chemical against *Alternaria solani

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 (Fig.1) 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 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) [11] proved the effectiveness of boric acid against *A. lunata* inciting fruit rot of tomato. Ashour (2009) [2] 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 1: *In vitro* efficacy of chemicals against *Alternaria solani*

T. 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	

1.2 *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 1 and depicted in the Table 2. 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) [14] 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.

PLATE- I (a)

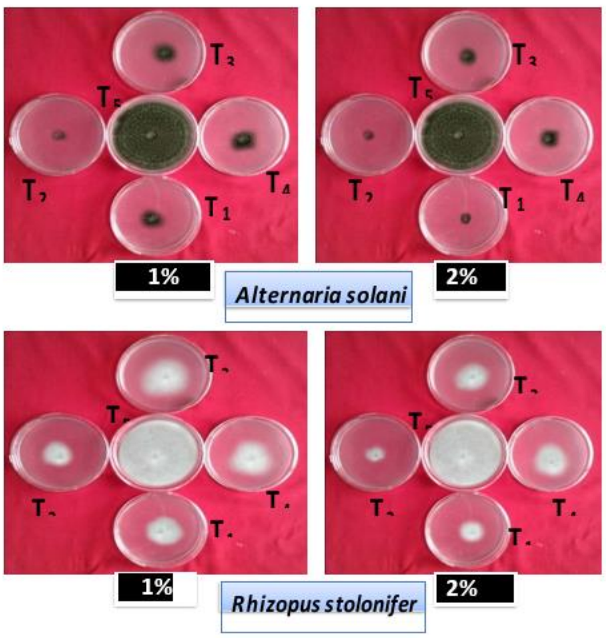

Fig 1: *In vitro* efficacy of different chemicals against post-harvest fungal diseases of *tomato*.

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

Table 2: *In vitro* efficacy of chemicals against *Rhizopus stolonifer*

T. 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	

In vitro* efficacy of chemical against *Aspergillus niger

Efficacy of five chemicals *viz*., Boric acid, Calcium chloride, Potassium chloride and sodium bicarbonate were tested using Poisoned Food Technique (PFT) for inhibition of mycelial growth of *Aspergillus niger*. Each chemical at concentrations of 1.0%, and 2.0% were tested separately for inhibition of mycelial growth. Results obtained on mycelial growth inhibition of *Aspergillus niger* with tested chemicals are presented in the Table .3. Results indicated that all the chemicals tested (@ 1% and 2% each) significantly inhibited mycelial growth of the test pathogen, over untreated control. At 1 per cent concentration of different chemicals, radial mycelia growth of *Aspergillus niger* was recorded from 2.00 mm to 10.75 mm as against 88.00 mm in untreated control. However, significantly least mycelial growth (2.00 mm) was observed by recording maximum inhibition of 97.72% with Calcium chloride which was at par with treatment of Boric acid (92.89%), while least mycelial inhibition was recorded with Sodium bicarbonate (88.06%) and Potassium chloride (87.78%). At 2 per cent concentration of different chemicals, radial mycelial growth *Aspergillus niger* was recorded from 01.25 mm to 09.75 mm as against 88.00 mm in untreated control. However, significantly least mycelial growth was observed by recording maximum inhibition (98.57%) with Calcium chloride which was at par with treatment of Boric acid (94.31%) while least mycelial inhibition was recorded with Sodium bicarbonate (90.62%) and Potassium chloride (88.92%). The results of present investigation resembling the findings of earlier workers, Tanaka and Nonaka (1981) [12] found that post-harvest bulb rot in onion was reduced 16 to 17 per cent when dusting of topped onions with calcium carbonate was carried out. Dang and Gupta (1984) [6] studied the chemical control of storage rot of onion bulbs caused by *Fusarium solani* and found that pre inoculation treatment with 2,4-D, potassium meta-bisulphite, diphenylamine and acetic acid gave 58 per cent reduction in rotting of onion bulbs.

Table 3: *In vitro* efficacy of chemicals against *Aspergillus niger*

T. No.	Treatment	Colony Dia.*(mm)	Per cent Inhibition	Colony Dia.*(mm)	Per cent Inhibition
		1%		2%	
T1	Boric acid	6.25	92.89	05.00	94.31
T2	Calcium Chloride	2.00	97.72	01.25	98.57
T3	Potassium Chloride	10.75	87.78	09.75	88.92
T4	Sodium Bicarbonate	10.50	88.06	08.25	90.62
T5	Control	88.00	00.00	88.00	00.00
	SE±	00.73		00.58	
	CD @ 1%	03.14		02.35	

In-vitro* efficacy of chemical against *Colletotrichum gloeosporioides

Efficacy of five chemicals *viz*., Boric acid, Calcium chloride, Potassium chloride and sodium bicarbonate were tested using Poisoned Food Technique (PFT) for inhibition of mycelial growth of *Colletotrichum gloeosporides*. 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 *Colletotrichum gloeosporioides* with tested chemicals are presented in the Table.4. Results indicated that all the chemicals tested (@ 1.0 % and 2.0 % each) significantly inhibited mycelial growth of the test pathogen, over untreated control [PLATE V (b)]. At 1 per cent concentration of different chemicals, radial mycelial growth *Colletotrichum gloeosporioides* was recorded from 10.50 mm to 16.25 mm as against 89.00 mm in untreated control. However, significantly least mycelial growth (10.50 mm) was observed by recording maximum growth inhibition was (88.20%) with Potassium chloride which was at par with treatment of Sodium bicarbonate (84.83%) and Boric acid (84.55%) while least mycelial inhibition was recorded with Calcium chloride (81.74%). At 2 per cent concentration of different chemicals, radial mycelial growth *Colletotrichum* *gloeosporioides* was recorded from 06.25 mm to 11.00 mm as against 89.00 mm in untreated control. However, significantly least mycelial growth (06.25 mm) was observed by recording maximum mycelial growth inhibition was (92.97%) with Calcium chloride which was at par with Potassium chloride (91.57%) and Sodium bicarbonate (89.88%) while least mycelial inhibition was recorded with Boric acid (87.64 %). The results of present investigation resembling the findings of earlier worker Stosic *et al.* (2014) evaluated and compare the effects of calcium chloride and calcium hydroxide on *in-vitro* mycelial growth, spore germinations and germ tube growth of *Colletotrichum acutatum, Colletotrichum gloeosporioides, Alternaria alternate and Penicillium expansum* and observed that fungal isolates grew similarly or stimulated in the presence of 1% and 1.5% calcium salts compared to the control, whereas noted reduction of mycelial growth only on PDA supplemented with 2% calcium salts.

Table 4: ***In vitro*** efficacy of chemicals against *Colletotrichum gloeosporioides*

T. No.	Treatment	Colony Dia.* (mm)	Per cent Inhibition	Colony Dia.* (mm)	Per cent Inhibition
		1%		2%	
T1	Boric acid	13.75	84.55	11.00	87.64
T2	Calcium Chloride	16.25	81.74	6.25	92.97
T3	Potassium Chloride	10.50	88.20	7.50	91.57
T4	Sodium Bicarbonate	13.50	84.83	9.00	89.88
T5	Control	89.00	00.00 (00.00)	89.00	00.00
	SE±	01.02		0.83	
	CD @ 1%	04.12		3.37	

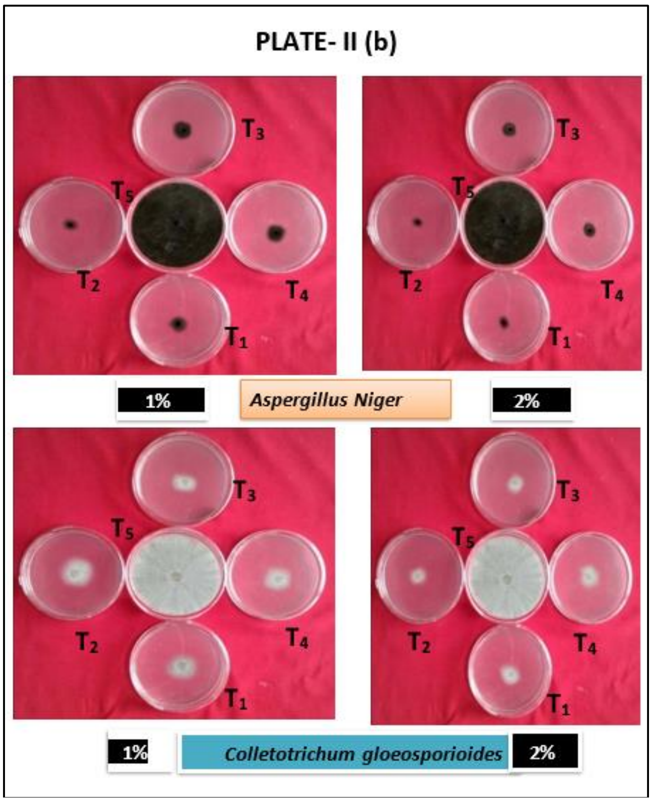

Fig 2: *In vitro efficacy* of different chemicals against post-harvest fungal diseases of tomato**.**

T_1 Boric acid **T_2 Calcium chloride**
T_3 Potassium Chloride **T_4 Sodium bicarbonate**
T_5 Control

Conclusion

All the four chemicals tested *in-vitro* were found to inhibit mycelial growth of *C. gloeosporioides, Alternaria solani, Aspergillus niger and Rhizopus stolonifer* However, Cacl2 and Boric acid, were highly effective *C. gloeosporioides, Alternaria solani, Aspergillus niger and Rhizopus stolonifer* as compared to all other treatments. All the tested chemicals were found fungistatic against *A. solani Aspergillus niger R. stolonifer* and *Colletotrichum gloeosporioides.* Calcium chloride and Boric acid at both concentration 1 & 2% were found most effective in inhibit the mycelial growth of *Alternaria solani, Aspergillus niger* and *Rhizopus stolonifer,* while least inhibition of these fungi was observed with Potassium chloride and Sodium bicarbonate *Colletotrichum gloeosporioides* gave least growth in treatment of Potassium chloride and Boric acid at 1% while at 2% Calcium chloride inhibited the maximum growth of *C. gloeosporioides* followed by Potassium chloride, while the least average mycelial inhibition was recorded with Sodium bicarbonate and Boric acid 87.64%.

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