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**DB Kolhe**
Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India

**RA Chavan**
Department of Plant Pathology, College of Agriculture, Osmanabad, Maharashtra, India

**RG Brahmankar**
Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India

**PA Sahane**
Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India

**RB Bade**
Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India

**Corresponding Author:**
**DB Kolhe**
Department of Plant Pathology, College of Agriculture, Latur, Maharashtra, India

# Efficacy of plant extracts and chemicals against *Rhizopus* fruit rot of sapota

**DB Kolhe, RA Chavan, RG Brahmankar, PA Sahane and RB Bade**

**Abstract**
*In vitro* efficacy of plant extracts and chemicals against *Rhizopus* rot of sapota" was studied at the Department of Plant Pathology, college of Agriculture, Latur (VNMKV, Parbhani), M.S. during the year 2019-20. Market samples of soft rot of sapota showing typical coarse grey colour growth yielded the growth of *Rhizopus stolonifer* and proved pathogenic causing similar symptoms. All plant extracts *viz*., Ginger, Turmeric, Neem, Garlic, Nilgiri, Onion and Tulsi and chemicals *viz*., Boric acid, Calcium chloride, Sodium bicarbonate and Potassium chloride were found effective in inhibition of mycelial growth of *Rhizopus stolonifer*. Garlic rhizome extract gave maximum mycelial growth inhibition (86%) followed by Nilgiri oil (85.15%), Turmeric rhizome extract (79.37%), Onion bulb extract (67.18%) and Tulsi leaf extract (62.71%). Least mycelial inhibition was recorded with Neem leaf extract (54.60%) and Ginger rhizome extract (52.41%). Among chemicals, Calcium chloride and Boric acid at concentration 0.5, 1 & 2% were found most effective in inhibiting the mycelial growth of *Rhizopus stolonifer*, least inhibition was observed with Potassium chloride and Sodium bicarbonate.

**Keywords:** Plant extract, chemicals, *in vitro*, sapota, *Rhizopus stolonifer*

## Introduction
Sapota (*Manilkara achras* L.), belongs to family sapotaceae and genus *Manikara* is popularly known as chiku in India. Fruit rot is one of the major factor in sapota cultivation causing rotting of sapotas by pathogens between harvest and consumption which ultimately make sapota fruits unfit for consumption. Among reported diseases *Rhizopus stolonifer* is major post harvest pathogen causing typical soft rot of sapota. It is essential need to develop new and effective methods of controlling post harvest diseases that are perceived as safe by the public and pose negligible risk to human health and the environment. Hence present investigation was conducted to study the *in vitro* efficacy of plant extracts and chemicals against *Rhizopus stolonifer* associated with fruit rot of sapota.

## Material and Methods
Present study entitled "*In vitro* efficacy of plant extracts and chemicals against *Rhizopus stolonifer* associated with fruit rot of sapota" was conducted at the Department of Plant Pathology College of Agriculture, Latur during the year 2019-20.

### Isolation of *Rhizopus stolonifer*
Diseased (rotten) sapota fruits were collected from different local marketing sites of Dist. Osmanabad. Randomly selected diseased fruits from each place were brought to the laboratory of department of Plant Pathology in clean polythene bags for isolation study. Isolations of collected diseased samples of sapota fruits were made on potato dextrose agar (PDA) medium by standard isolation method. Growths of fungi were obtained within 2 to 3 days in all petri plates and fungus was identified on morphological character as *Rhizopus stolonifer*.

### *In vitro* evaluation of plant extract against *Rhizopus stolonifer* associated with sapota
Efficacy of different plant extracts *viz*., Ginger, Turmeric, Neem, Garlic, Nilgiri, Onion and Tulsi for the possible presence of antifungal properties against *Rhizopus stolnifer* associated with sapota rot was tested through by Poisoned Food Technique.
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. Three plates for each treatment were maintained along one set of 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 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).

$$X = \frac{Y - Z}{Y} \times 100$$

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 major fungi associated with sapota rot

| Sr. No. | Name of Plant extracts | Common name | Family | Plant Part used | Conc. (%) |
|---|---|---|---|---|---|
| 1 | *Zingiber officinal* | Ginger | Zingiberaceae | Rhizome | 10% |
| 2 | *Curcuma longa* | Turmeric | Zingiberaceae | Rhizome | 10% |
| 3 | *Azadirachta indica* | Neem | Meliaceae | Leaves | 10% |
| 4 | *Allium sativum* | 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 | | | | |

### *In vitro* efficacy of chemicals against major fungi associated with post harvest rot of sapota

The effect of Boric acid, Calcium chloride, Sodium bicarbonate and Potassium chloride were tested *In vitro* separately at 0.5%, 1% and 1.5% concentrations (Table 2) on mycelial growth of major fungi associated with sapota rot. Each chemical was amended to PDA medium at a required concentration of 0.5%, 1% and 1.5%.

Petri plates containing PDA without chemicals were served as control. Four replication were maintained for each treatment. After medium solidification plates were inoculated with 5 mm discs of 7-day-old culture of each test fungus and incubated at 25 ± 2 $^{O}$C for 7 days. The colony diameters of the fungal pathogens on medium were recorded and per cent inhibition over control was calculated by the following formula of Horsfall (1956) [2].

$$X = \frac{Y - Z}{Y} \times 100$$

Where, X = Per cent inhibition,
Y = Growth of fungus in control (mm)
Z = Growth of fungus in treatment (mm)

**Table 2:** List of chemicals used against major fungi associated with sapota rot

| Sr. No. | Name of chemicals | Concentration (%) |
|---|---|---|
| 1. | Boric acid | 0.5, 1 and 1.5 |
| 2. | Calcium chloride | 0.5, 1 and 1.5 |
| 3. | Potassium chloride | 0.5, 1 and 1.5 |
| 4. | Sodium bicarbonate | 0.5, 1 and 1.5 |
| 5. | Control | |

## Result and discussion

### *In vitro* efficacy of plant extracts against *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. Result (Table 3, PLATE I & Fig. 1) revealed that radial mycelial growth of *Rhizopus stolonifer* was recorded from 12.60 mm to 42.83 mm as against 90.00 mm in untreated control. However, significantly least mycelial growth was recorded with maximum inhibition of 86% with treatment of Garlic rhizome extract followed by Nilgiri oil (85.15%), Turmeric rhizome extract (79.37%), Onion bulb extract (67.18%) and Tulsi leaf extract (62.71%). While least mycelial inhibition was recorded with Neem leaf extract (54.60%) and Ginger rhizome extract (52.41%).

The results of present investigation resembling the findings of earlier workers *viz*., Un- Nisa *et al*. (2010) [6] who tested efficacy of plant extracts on spore germination of *Rhizopus stolonifer* and *Alternaria alternate* and showed that extract of *Allium sativum* was highly effective followed by *Allium cepa* and *Mentha avensis* in inhibition of spore germination. Oladele (2019) [5] tested antifungal activity of garlic powder against mycelial growth of three post-harvest pathogens *Aspergillus*, *Rhizopus* and *Mucor* by different weight and showed that the different weights of the garlic powder apart from the control (0 g garlic) significantly inhibited the mycelial growth of the three post-harvest pathogens. Bhalerao *et al.* (2019) [1] evaluated seven plant extracts *In vitro* against *Rhizopus stolonifer* and reported that least mycelial growth was recorded in Nilgiri oil followed by Garlic clove extract.


**Plate I:** *Rhizops stolonifer*

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

| Tr. No. | Colony Dia. (mm) | Per cent Inhibition |
|---|---|---|
| $T_1$ | 42.83 | 52.41 |
| $T_2$ | 18.56 | 79.37 |
| $T_3$ | 40.86 | 54.60 |
| $T_4$ | 12.60 | 86.00 |
| $T_5$ | 13.36 | 85.15 |
| $T_6$ | 29.53 | 67.18 |
| $T_7$ | 33.56 | 62.71 |
| $T_8$ | 90.00 | |
| SE± | 0.51 | |
| CD @ 1% | 1.51 | |

Colony diameter = Average of three replications

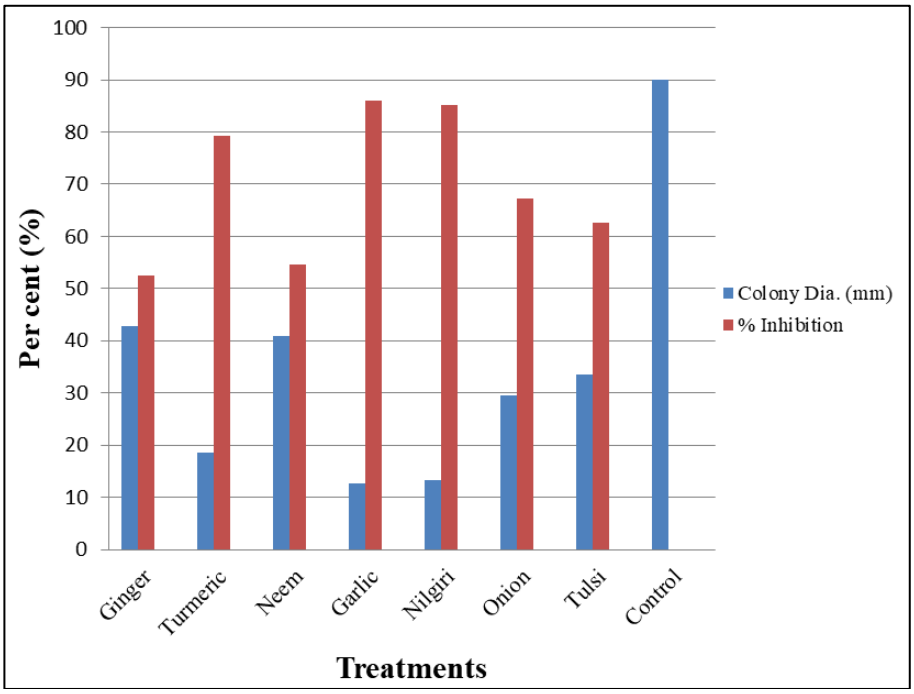


**Fig 1:** *In vitro* efficacy of plant extracts against *Rhizopus stolonifer*

***In vitro* efficacy of chemical against *Rhizopus stolonifer***

Efficacy of four chemicals *viz*., Boric acid, Calcium chloride, Potassium chloride and sodium bicarbonate at various concentrations of 0.5%, 1.0% and 1.5% were tested separately for inhibition of mycelial growth using Poisoned Food Technique (PFT). Results indicated that all the chemicals tested (@ 0.5%, 1.0% and 1.5% each) significantly inhibited mycelial growth of the test pathogen, over untreated control. (Table 4, fig. 2 and PLATE II)

At 0.5 per cent concentration, radial mycelial growth *Rhizopus stolonifer* was recorded from 27.37 mm to 46.22 mm as against 90 mm in untreated control. However, significantly least mycelial growth (27.37 mm) was observed by recording maximum inhibition of 69.58% with Calcium chloride followed by Boric acid (64.33%) While least mycelial inhibition was recorded with Potassium chloride (53.22%) and Sodium bicarbonate (48.64%).

At 1 per cent concentration of chemicals, radial mycelial growth *Rhizopus stolonifer* was recorded from 13.55 mm to 30.80 mm as against 90 mm in untreated control. However, significantly least mycelial growth (13.55 mm) was observed by recording maximum inhibition of 89.94% with Calcium chloride followed by Boric acid (74.86%), while least mycelial inhibition was recorded with Potassium chloride (69.31%) and Sodium bicarbonate (65.77%).

At 1.5 per cent concentration of chemicals, radial mycelial growth *Rhizopus stolonifer* was recorded from 7.57 mm to 22.47 mm as against 90 mm in untreated control. However, significantly least mycelial growth (7.57 mm) was observed by recording maximum inhibition of 91.58% with Calcium chloride followed by boric acid (89.88%), while least mycelial inhibition was recorded with Potassium chloride (78.75%) and Sodium bicarbonate (75.03%).

The results of present investigation resembling the findings of earlier workers *viz.*, Ismail *et al*. (2010) [3] reported that Calcium chloride (2%) reduced the linear growth of *Rhizopus stolonifer*. Nadia *et al*. (2014) [4] reported that Potassium sorbate gave complete mycelial growth inhibition of *R. stolonifer* (90.00%) on PDA media followed by Calcium chloride (80.99%). Bhalerao *et al*. (2019) [1] reported that $Cacl_2$ and Boric acid were highly effective in inhibition of mycelial growth of *Rhizopus stolonifer* and *Aspergillus niger*.


0.5% 1% 1.5%

**Plate II:** *Rhizopus stolonifer*

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

| Tr. No. | Colony Dia. (mm) | Per cent Inhibition | Colony Dia. (mm) | Per cent Inhibition | Colony Dia. (mm) | Per cent Inhibition |
|---|---|---|---|---|---|---|
| | 0.5% | | 1% | | 1.5% | |
| $T_1$ | 32.10 | 64.33 | 22.62 | 74.86 | 9.10 | 89.88 |
| $T_2$ | 27.37 | 69.58 | 13.55 | 89.94 | 7.57 | 91.58 |
| $T_3$ | 42.10 | 53.22 | 27.62 | 69.31 | 19.12 | 78.75 |
| $T_4$ | 46.22 | 48.64 | 30.80 | 65.77 | 22.47 | 75.03 |
| $T_5$ | 90.00 | | 90.00 | | 90.00 | |
| SE± | 0.51 | | 0.42 | | 0.34 | |
| CD @ 1% | 1.48 | | 1.22 | | 0.98 | |

Colony diameter = Average of four replications

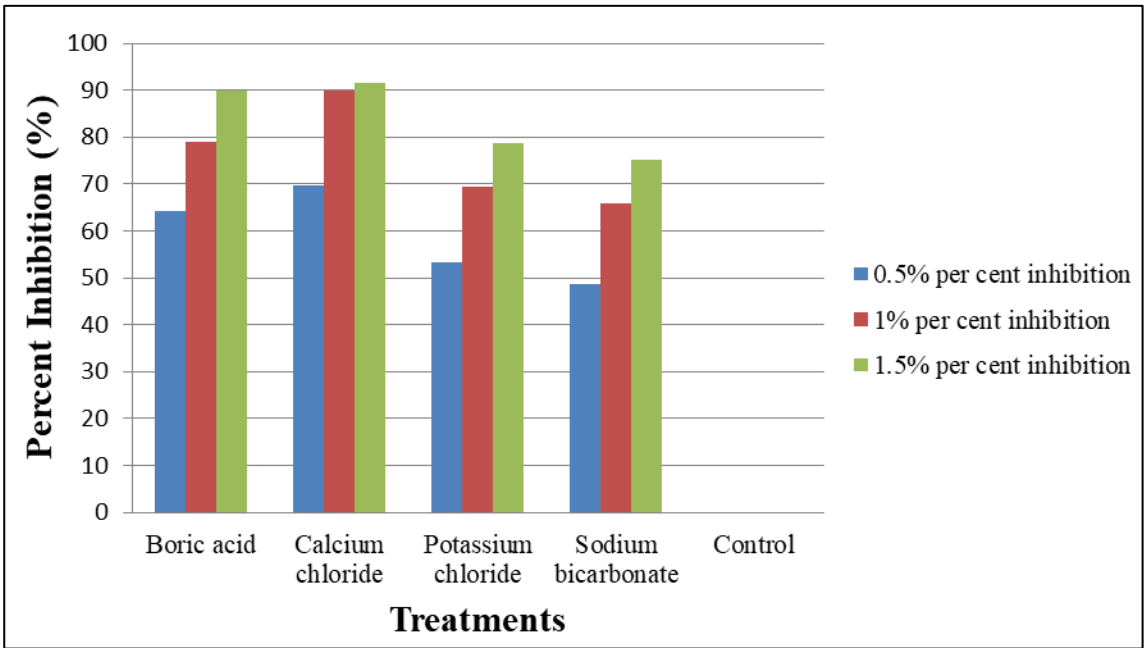


**Fig 2:** *In vitro* efficacy of chemicals against *Rhizopus stolonifer*

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