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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

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

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

**VB Udar**
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 *Aspergillus* fruit rot of sapota

**DB Kolhe, RA Chavan, PA Sahane, RG Brahmankar and VB Udar**

**Abstract**
Present research work entitled "Studies on *In vitro* efficacy of plant extracts and chemicals against *Aspergillus* rot of sapota" was conducted at the Department of Plant Pathology, College of Agriculture, Latur (VNMKV, Parbhani), M.S. during the year 2019-20. The present investigation was carried out to evaluate *in vitro* efficacy of different chemicals *viz*., Boric acid, Calcium chloride, Sodium bicarbonate and Potassium chloride and plant extracts *viz*., Ginger, Turmeric, Neem, Garlic, Nilgiri, Onion and Tulsi against *Aspergillus niger* causing typical sapota rot. All plant extracts were effective against *Aspergillus niger*, however least mycelial growth was recorded with per cent inhibition with treatment of Garlic clove extract (100%) followed by Ginger rhizome extract (90.37%), Turmeric (89.55%), Neem leaf extract (88.33%), Nilgiri oil (85.26%). Least mycelial inhibition was recorded with Onion bulb extract (77.60%) and Tulsi leaf extract (17.96%). All the tested chemicals were found fungistatic against *Aspergillus niger*. Calcium chloride and Boric acid at concentration 0.5, 1 & 2% were found most effective in inhibiting the mycelial growth of *Aspergillus niger*, while least inhibition of these fungi was observed with Sodium bicarbonate and Potassium chloride.

**Keywords:** Plant extract, chemicals, sapota rot, *Aspergillus niger*

## Introduction
Sapota (*Manilkara achras* L.), a tropical evergreen fruit crop belongs to family sapotaceae is one of the important fruit crop in the state of Maharashtra and also grown on commercial scale in Marathwada region. Fruit rot is one of the major hurdle in sapota cultivation causing rotting of sapotas between harvest and consumption which ultimately make sapota fruits unfit for consumption. Several fungal diseases like sour rot (*Geotrichum candidum*), *Cladosporium* rot (*Cladosporium oxysporum*), blue mould rot (*Penicillium itallicum*), black mould rot (*Aspergillus niger*), *Rhizopus* rot etc (Bakar and Karim, 1990) [1] were reported as post-harvest pathogens of sapota. Among reported diseases *Aspergillus niger* is major post-harvest pathogen causing typical black rot with watery secretion and emittence of odour. Present investigation was undertaken to full fill an urgent need of development of 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, evaluated plant extracts and chemicals *in vitro* against *Aspergillus niger* associated with fruit rot of sapota.

## Material and methods
### Collection of sapota fruits affected with *Aspergillus* rot
Present research work entitled "*in vitro* efficacy of plant extracts and chemicals against *Aspergillus* rot of sapota" was conducted at the Department of Plant Pathology College of Agriculture, Latur during the year 2019-20.Typical soft black rotten sapota fruits were collected from local marketing sites of Dist. Osmanabad and were brought to the laboratory of department of Plant Pathology in clean polythene bags for isolation.

### Isolation of *Aspergillus niger*
Isolations were made from diseased fruits on potato dextrose agar (PDA) medium. Growth of isolated fungus was obtained within 3 to 5 days and bits of small mycelial growth from the typical colonies were transferred on slants of PDA under aseptic condition and identified as *Aspergillus niger* on morphological character.

### *In vitro* evaluation of plant extract against *Aspergillus niger*
The present investigation was carried out to evaluate the efficacy of different plant extracts *viz*., Ginger, Turmeric, Neem, Garlic, Nilgiri, Onion and Tulsi for the possible presence of antifungal properties against *Aspergillus niger* causing sapota rot 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. Plant extracts with desired concentration were incorporated before solidification of media 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. 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) [3].

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

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 *Aspergillus* fruit rot of sapota

| 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* 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 | | | | |

***In vitro* efficacy of chemicals against *Aspergillus niger***

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 on mycelial growth of *Aspergillus niger* associated with sapota rot (Table 2). 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 ± 2OC for 7 days. The colony diameters of the fungal pathogens were measured and per cent inhibition over control was calculated by the following formula of Horsfall (1956) [3].

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

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 *Aspergillus* fruit rot of sapota

| 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 *Aspergillus niger***

Efficacy of seven plant extracts were tested using Poisoned Food Technique (PFT) for inhibition of mycelial growth of *Aspergillus niger*. Evaluation of different plant extracts for their fungitoxic properties against *Aspergillus niger* showed significant inhibition of growth of test pathogen *In vitro* over control. Result (Table 3 & PLATE I) revealed that radial mycelial growth of *Aspergillus niger* was recorded from 0.00 mm to 73.83 mm as against 90 mm in untreated control. However, significantly least mycelial growth was recorded with maximum inhibition of 100% with treatment of Garlic clove extract followed by Ginger rhizome extract (90.37%), Turmeric (89.55%), Neem leaf extract (88.33%), Nilgiri oil (85.26%). While least mycelial inhibition was recorded with Onion bulb extract (77.60%) and Tulsi leaf extract (17.96%).

The results of present investigation resembling the findings of earlier workers *viz*., Patel *et al*. (2008) [4] reported that the extract from *Allium sativum* was significantly better followed by extract of *Jatropa curcas, Aloe bar badensis* and *Azrdirachta indica* in controlling *Aspergillus niger* causing anola fruit rot. Mohamed *et al*. (2012) [7] observed that garlic cloves, onion and neem leaf extracts had the ability to cause reduction in the mycelial growth of *A. niger*. Pudake *et al*. (2018) [5] who also reported the effectiveness of garlic bulbs and nilgiri extract in complete inhibition of mycelium growth of *Aspergillus niger*.

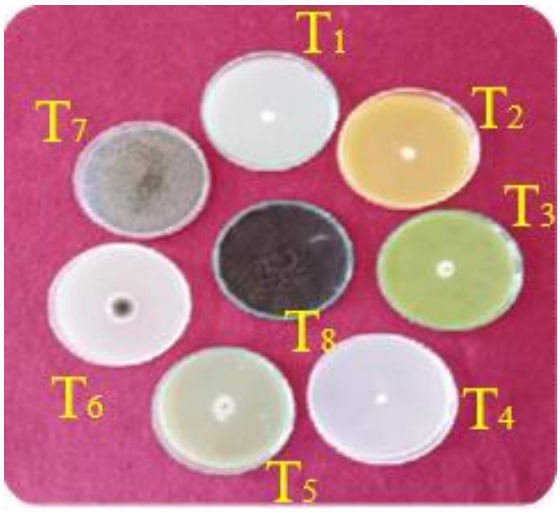


**Plate I:** *In vitro* efficacy of plant extracts against *Aspergillus niger*

**Table 3:** *In vitro* efficacy of plant extracts against *Aspergillus niger*

| Tr. No. | Colony Dia. (mm) | Per cent Inhibition |
|---|---|---|
| $T_1$ | 8.66 | 90.37 |
| $T_2$ | 9.40 | 89.55 |
| $T_3$ | 10.50 | 88.33 |
| $T_4$ | 0.00 | 100 |
| $T_5$ | 13.26 | 85.26 |
| $T_6$ | 20.16 | 77.60 |
| $T_7$ | 73.83 | 17.96 |
| $T_8$ | 90.00 | |
| SE± | 0.47 | |
| CD @ 1% | 1.38 | |

Colony diameter = Average of three replications

### *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 0.5%, 1.0% and 1.5% were tested separately for inhibition of mycelial growth. Results (Table 4 and Plate II) indicated that all the chemicals tested at all concentration were found effective in inhibition of mycelial growth of the test pathogen, over untreated control.

At 0.5 per cent concentration of different chemicals, radial mycelial growth of *Aspergillus niger* was recorded from 12.97 mm to 29.62 mm as against 90 mm in untreated control. However, significantly least mycelial growth (12.97 mm) was observed by recording maximum inhibition of 85.58% with Calcium chloride followed by Boric acid (82.42%).

At 1 per cent concentration, radial mycelial growth *Aspergillus niger* was recorded from 10.37 mm to 22.57 mm and least mycelial growth with maximum inhibition (88.47%) was observed with Calcium chloride followed by Boric acid (85.16%).

At 1.5 per cent concentration of different chemicals, radial mycelial growth *Aspergillus niger* was recorded from 5.80 mm to 12.10 mm, However, significantly least mycelial growth with maximum inhibition (93.55%) was observed with Calcium chloride followed by Boric acid (91.36%).

At all concentration least mycelial inhibition was observed with Sodium bicarbonate and Potassium chloride.

Earlier investigation also indicated the effectiveness of inorganic chemicals in arresting the mycelial growth of *Aspergillus*. Thanh Toan *et al*. (2018) [6] reported that 20 mM $CaCl_2$ was the most effective concentration in antifungal assay to *Aspergillus* isolated from orange rot. Bhalerao *et al*. (2019) [2] reported that $Cacl_2$ and boric acid were highly effective in inhibition of mycelial growth of *Rhizopus stolonifer* and *Aspergillus niger*.

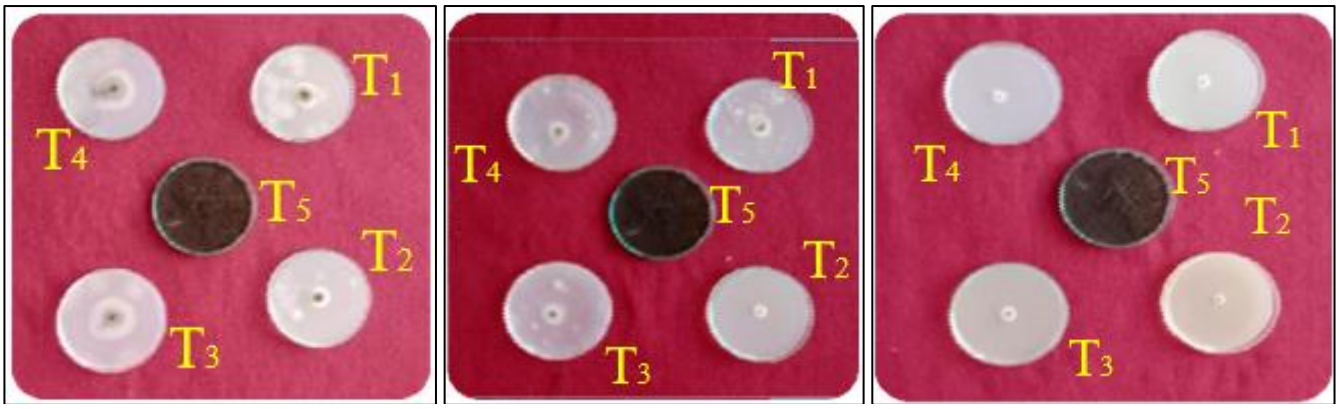


**Plate II:** *In vitro* efficacy of chemical against *Aspergillus niger*

**Table 4:** *In vitro* efficacy of chemicals against *Aspergillus niger*

| 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% | |
| $T_1$ | 15.82 | 82.42 | 13.35 | 85.16 | 7.77 | 91.36 |
| $T_2$ | 12.97 | 85.58 | 10.37 | 88.47 | 5.80 | 93.55 |
| $T_3$ | 29.62 | 67.08 | 22.57 | 74.92 | 12.10 | 86.55 |
| $T_4$ | 25.65 | 71.5 | 18.62 | 79.31 | 9.62 | 89.31 |
| $T_5$ | 90.00 | | 90.00 | | 90.00 | |
| SE± | 0.39 | | 0.29 | | 0.41 | |
| CD @ 1% | 1.13 | | 0.84 | | 1.18 | |

Colony diameter = Average of four replications

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