

## *In vitro* evaluation of botanicals against post harvest disease causing pathogens of mandarin orange

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**Abstract:** Isolation carried out from infected mandarin fruits and recorded association of three fungi viz; *Aspergillus niger*, *Penicillium digitatum* and *Rhizopus stolonifer* and pathogenicity of all three isolates were proved. The pathogen *Aspergillus niger* produced brown to black lesions on fruits, *Penicillium digitatum* produced greenish spots on fruits while *Rhizopus stolonifer* produced blackening thereafter inducing severe rotting. Ten Botanicals were tested against these tree pathogens at 5% and 10% concentrations, among these botanicals 10% concentration of all herbal extracts was effective against all three pathogens except *Aloe vera*.

**Key words:** Mandarin, isolation, botanicals, post harvest.

### INTRODUCTION

Orange (*Citrus reticulata* Blanco) is most common among citrus fruits in India and occupies nearly forty percent of the total area under citrus cultivation. India is the third largest producer of orange in the world. Although, India is second in area and third in production of orange in the world, the productivity/hectare is very low as compared to the US, Indonesia, Turkey and other countries where the crop is grown commercially. The area under orange cultivation in India is 31.12 mha and the production is 290.63 mt during 2013. In terms of productivity, India ranks 64<sup>th</sup> in the world with only 9.23 t/ha. Maharashtra is the leading orange (Mandarin) producing State with 37.0 mt production in 2013 accounting for 40% of total production with productivity of 2.8 mt/ha. The area under cultivation of mandarin orange in Maharashtra is 13.3 mha (Anonymous, 2013). The seasonal disease profile of mandarin orange fruit

clearly marked two distinct peak periods of fungal rot spoilage. In the first peak, the major monthly fruit rot loss was inflicted by green and blue mold rots caused by *Penicillium digitatum* (1.78-2.44%) and *P. italicum* (0.73-1.30%). The second peak period of fruit spoilage inflicted by *Aspergillus niger* was observed during the summer months of May (5.27%) and June (6.05%). The cumulative rottage losses during the entire marketing season were 34.30%. Several species and varieties of citrus are subjected to various diseases caused by fungi, bacteria, viruses, phytoplasma and other pathogenic entities. In fungal post harvest diseases of mandarin (*Citrus reticulata* Blanco), fruit spoilage or decay after harvest is caused by many fungal pathogens such as *Penicillium italicum* and *Penicillium digitatum* causing green mold and blue mold, black core rot by *Aspergillus spp*, *Rhizopus* rot by *Rhizopus spp*. Most of these fungal pathogens are carried out from the orchard soils,

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contaminated handling practices and storage conditions after harvest.

## MATERIAL AND METHODS

### Isolation of pathogens associated with infected fruits of mandarin orange

The micro-organisms responsible for spoilage of mandarin fruits were isolated on potato dextrose agar medium by employing tissue isolation method. Fungi isolated from diseased samples were identified on the basis of morphological characters observed under microscope and identified as *Aspergillus*, *Penicillium* and *Rhizopus*.

### Bio-efficacy of plant extracts against test pathogens

The antagonistic potential of botanicals like Babul (*Acacia arabica* wild), Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Kanher (*Nerium indicum*), Nilgiri (*Eucalyptus spp.*), Zandu (*Tagetes spp.*), Onion (*Allium sepa*), Tulas (*Ocimum sanctum*), Korphad (*Aloe vera*) and Sadaphuli (*Vinca rosea*) were tested against the pathogen causing post harvest diseases in mandarin viz., *Aspergillus*, *Penicillium*, and *Rhizopus*.

For preparation of plant extract, fresh leaves/bulbs of each selected botanicals as mentioned earlier were collected from Dhanvantari, Central Campus M.P.K.V., Rahuri and washed under tap water before use. The 60 gm fresh sample of each botanical except *Aloe vera* was crushed in mortar and pestle with 150 ml 70% Ethanol and Acetone solvent was used for extraction of juice from *Aloevera* in same concentration as ethanol. The ground extract was filtered through double layer muslin cloth. Thereafter the plant extract solution was filtered through G-4 bacterial filter. Thus filtered extract was used as stock solution and evaluated against the test pathogen at 5% and 10% concentration following poison food technique (Nene and Thapliyal 1982). Two liters PDA media was prepared and distributed evenly (100ml/ flask) in 200 ml capacity conical flask and sterilized at 1.05 kg/cm<sup>2</sup> pressure for 15 minutes. The measured quantity of (10% i.e., 10 ml extract in 100 ml media) each botanical was added in each flask separately before pouring. The flask

were shaken thoroughly and poured into three petri plates. The petri plates containing 10 % botanical extract were inoculated with 5 mm fungal disc of seven days old culture grown on PDA and incubated at 27±1°C in BOD incubator for 7 days. Each treatment of botanicals was repeated thrice. The plates of PDA without botanicals and inoculated with test pathogen separately served as control. The observations on colony diameter were recorded on 7 days after inoculation and per cent inhibition of mycelia growth over control was calculated by using formula,

$$I = \frac{100(C - T)}{C}$$

Where, I = Per cent inhibition of fungal growth.

C = Growth of the test fungus in control.

T = Growth of test fungus in treatment.

## RESULTS AND DISCUSSION

### In vitro evaluation of botanicals against post harvest disease causing pathogens

The efficacy of 10 botanicals viz., Garlic (*Allium sativum*), Nilgiri (*Eucalyptus spp.*), Onion (*Allium sepa*), Korphad (*Aloe vera*), Kanher (*Nerium indicum*), Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), Babul (*Acacia sativum*), Zandu (*Tagetes spp.*) and Sadaphuli (*Vinca rosea* Linn) were evaluated *In vitro* for control of these post harvest pathogens of mandarin orange under present investigations and the results are presented in Tables 1, 2, and 3.

#### 1. *Aspergillus niger*

The results of evaluation of botanicals against *Aspergillus niger* *In vitro* are presented in Table 1a. Significant differences were observed amongst different botanicals evaluated at 5% concentration. All the botanicals showed significantly low colony diameter as compared to control treatment after 8 days. It was observed that among ten botanicals tried Kanher extract at 5% concentration was effective to inhibit *Aspergillus niger* (89.07%). It was on par with Nilgiri (88.88%), Sadaphuli (88.88%), Zandu (88.88%), Babul (88.88%), Garlic (88.88%) and Neem (87.03%) being not differing statistically from

each other. The botanicals like Aloe vera (52.96%) and Onion (61.11%) was less effective to inhibit the growth of fungus. Subsequently when all these ten botanicals tested at 10% concentration, almost all botanicals were effective to inhibit *Aspergillus niger* except *Aloe vera* (1.85%) (Table 1b).

The growth and sporulation of pathogen was poor to medium in the 5% concentration of botanical extract treatment while no growth and sporulation of pathogen was observed at 10% concentration except *Aloe vera* wherein poor growth and sporulation were recorded.

**Table 1a**  
In vitro evaluation of effect of 5% herbal extract over growth and sporulation of pathogen, *Aspergillus niger*

| Sr. no. | Herbal extract | Mean colony diameter (mm) | Per cent inhibition over control | Growth | Sporulation |
|---------|----------------|---------------------------|----------------------------------|--------|-------------|
| 1       | Garlic         | 10.00                     | 88.88                            | ++     | ++          |
| 2       | Onion          | 35.00                     | 61.11                            | +++    | +++         |
| 3       | Aloe vera      | 42.33                     | 52.96                            | +++    | +++         |
| 4       | Kanher         | 9.83                      | 89.07                            | ++     | ++          |
| 5       | Nilgiri        | 10.00                     | 88.88                            | ++     | ++          |
| 6       | Sadafuli       | 10.00                     | 88.88                            | ++     | ++          |
| 7       | Zandu          | 10.00                     | 88.88                            | ++     | ++          |
| 8       | Neem           | 11.67                     | 87.03                            | ++     | ++          |
| 9       | Babul          | 10.00                     | 88.88                            | ++     | ++          |
| 10      | Tulsi          | 13.33                     | 85.18                            | ++     | ++          |
| 11      | Control        | 90.00                     | 00.00                            | ++++   | ++++        |
|         | SE (±)         | 1.20                      |                                  |        |             |
|         | CD @ 1 %       | 4.81                      |                                  |        |             |

- = No growth, ++ = Poor +++ = Medium, , ++++ = Good

Thus, the results indicated that 10% concentration of extract of all the botanicals was effective against *Aspergillus niger* than 5% concentration. Whereas herbal extract of Aloe vera was not effective against the pathogen.

## 2. *Penicillium digitatum*

The results of evaluation of botanicals against *Penicillium digitatum* in vitro presented in Table 2a indicated significant differences amongst different botanicals evaluated at 5% concentration. All the botanicals showed significantly low colony

**Table 1b**  
In vitro evaluation effect of 10% herbal extract over growth and sporulation of pathogen, *Aspergillus niger*

| Sr. No. | Herbal extract treatment at 10% concentration | Mean colony diameter (mm) | Per cent inhibition over control | Growth | Sporulation |
|---------|---|---------------------------|----------------------------------|--------|-------------|
| 1       | Garlic  | 0.00                      | 100.00                           | -      | -           |
| 2       | Onion   | 0.00                      | 100.00                           | -      | -           |
| 3       | Aloe vera                                     | 88.33                     | 1.85                             | ++     | ++          |
| 4       | Kanher  | 0.00                      | 100.00                           | -      | -           |
| 5       | Nilgiri                                       | 0.00                      | 100.00                           | -      | -           |
| 6       | Sadafuli                                      | 0.00                      | 100.00                           | -      | -           |
| 7       | Zandu   | 0.00                      | 100.00                           | -      | -           |
| 8       | Neem  | 0.00                      | 100.00                           | -      | -           |
| 9       | Babul   | 0.00                      | 100.00                           | -      | -           |
| 10      | Tulsi   | 0.00                      | 100.00                           | -      | -           |
| 11      | Control                                       | 90.00                     | 00.00                            | -      | -           |
|         | SE (±)  | 0.50                      |                                  |        |             |
|         | CD@1%   | 2.00                      |                                  |        |             |

- = No growth, ++ = Poor +++ = Medium, , ++++ = Good

diameter as compared to control treatment in the observation recorded after 8 days. It was observed that among 10 botanicals tried, Garlic extract at 5% concentration showed maximum inhibition of pathogen (68.81 %). Onion showed less inhibition (1.85 %) of pathogen growth.

Subsequently when all these ten botanicals when tested at 10% concentration, almost all botanicals showed 100% inhibition of *Penicillium digitatum* except Aloe vera which showed (87.03 %) inhibition of pathogen growth (Table 2b).

The growth and sporulation of pathogen was poor to medium in the 5% concentration of botanical extract treatment while no growth and sporulation of pathogen were observed at 10% concentration except *Aloe vera*.

Thus, the results indicated that 10% concentration of extracts of all the botanicals was effective against the post harvest pathogen *Penicillium digitatum* than at 5% concentration. Herbal extract of *Aloe vera* was not effective against the pathogen.

**Table 2a**

**In vitro evaluation effect of 5% herbal extract over growth and sporulation of pathogen, *Penicillium digitatum*.**

| Sr. no. | Herbal extract treatment at 5% concentration | Mean colony diameter (mm) | Per cent inhibition over control | Growth | Sporulation |
|---------|--|---------------------------|----------------------------------|--------|-------------|
| 1       | Garlic                                       | 31.67                     | 68.81                            | ++     | ++          |
| 2       | Onion  | 88.33                     | 1.85                             | ++++   | ++++        |
| 3       | Aloevera                                     | 90.00                     | 00.00                            | ++++   | ++++        |
| 4       | Kanher                                       | 90.00                     | 00.00                            | ++++   | ++++        |
| 5       | Nilgiri                                      | 90.00                     | 00.00                            | ++++   | ++++        |
| 6       | Sadafuli                                     | 90.00                     | 00.00                            | ++++   | ++++        |
| 7       | Zandu  | 90.00                     | 00.00                            | ++++   | ++++        |
| 8       | Neem   | 90.00                     | 00.00                            | ++++   | ++++        |
| 9       | Babul  | 90.00                     | 00.00                            | ++++   | ++++        |
| 10      | Tulsi  | 90.00                     | 00.00                            | ++++   | ++++        |
| 11      | Control                                      | 90.00                     | 00.00                            | ++++   | ++++        |
|         | SE (±)                                       | 0.71                      |                                  |        |             |
|         | CD@1%  | 2.83                      |                                  |        |             |

- = No growth, ++ = Poor, +++ = Medium, , +++++ = Good

**Table 2b**

**In vitro evaluation of effect of 10% herbal extract over growth and sporulation of pathogen, *Penicillium digitatum*.**

| Sr. No. | Herbal extract treatment at 5% concentration | Mean colony diameter (mm) | Per cent inhibition over control | Growth | Sporulation |
|---------|--|---------------------------|----------------------------------|--------|-------------|
| 1       | Garlic                                       | 0.00                      | 100.00                           | -      | -           |
| 2       | Onion  | 0.00                      | 100.00                           | -      | -           |
| 3       | Aloevera                                     | 11.67                     | 87.03                            | ++     | ++          |
| 4       | Kanher                                       | 0.00                      | 100.00                           | -      | -           |
| 5       | Nilgiri                                      | 0.00                      | 100.00                           | -      | -           |
| 6       | Sadafuli                                     | 0.00                      | 100.00                           | -      | -           |
| 7       | Zandu  | 0.00                      | 100.00                           | -      | -           |
| 8       | Neem   | 0.00                      | 100.00                           | -      | -           |
| 9       | Babul  | 0.00                      | 100.00                           | -      | -           |
| 10      | Tulsi  | 0.00                      | 100.00                           | -      | -           |
| 11      | Control                                      | 90.00                     | 00.00                            | -      | -           |
|         | SE (±)                                       | 0.50                      |                                  |        |             |
|         | CD @ 1%                                      | 2.00                      |                                  |        |             |

- = No growth, ++ = Poor, +++ = Medium, +++++ = Good

### 3. *Rhizopus stolonifer*

The results of evaluation of botanicals against *Rhizopus stolonifer* in vitro presented in Table 3a showed significant differences amongst different botanicals evaluated at 5% concentration. All the botanicals showed significantly low colony diameter as compared to control treatment in the observation recorded after 8 days. It was observed that among 10 botanicals tried, Tulsi extract at 5% concentration showed maximum inhibition (94.44%) of fungus which was on par with the treatment of Nilgiri (88.88%), Kanher (88.88%), Zendu (88.88%), Babul (88.88%), Neem (88.88%) and Garlic (87.03%). Onion was less effective in inhibiting the growth of fungus (9.25%) whereas *Aloe vera* failed to inhibit the growth of pathogen.

Subsequently when all these ten botanicals tested at 10% concentration, almost all botanicals showed 100% inhibition of *Rhizopus stolonifer*. *Aloe vera* showed less inhibition (85.18 %) of pathogen growth (Table 3b).

**Table 3a**

**In vitro evaluation effect of 5% herbal extract over growth and sporulation of pathogen, *Rhizopus stolonifer*.**

| Sr. no. | Herbal extract treatment at 5% concentration | Mean colony diameter (mm) | Per cent inhibition over control | Growth | Sporulation |
|---------|--|---------------------------|----------------------------------|--------|-------------|
| 1       | Garlic                                       | 11.67                     | 87.03                            | ++     | ++          |
| 2       | Onion  | 81.67                     | 9.25                             | ++++   | ++++        |
| 3       | Aloevera                                     | 90.00                     | 00.00                            | ++++   | ++++        |
| 4       | Kanher                                       | 10.00                     | 88.88                            | ++     | ++          |
| 5       | Nilgiri                                      | 10.00                     | 88.88                            | ++     | ++          |
| 6       | Sadafuli                                     | 90.00                     | 00.00                            | ++++   | ++++        |
| 7       | Zandu  | 10.00                     | 88.88                            | ++     | ++          |
| 8       | Neem   | 10.00                     | 88.88                            | ++     | ++          |
| 9       | Babul  | 10.00                     | 88.88                            | ++     | ++          |
| 10      | Tulsi  | 5.00                      | 94.44                            | ++     | ++          |
| 11      | Control                                      | 90.00                     | 00.00                            | ++++   | ++++        |
|         | SE (±)                                       | 0.71                      |                                  |        |             |
|         | CD@1%  | 2.83                      |                                  |        |             |

- = No growth, ++ = Poor, +++ = Medium, +++++ = Good

The growth and sporulation of pathogen were poor to medium in the 5% concentration of botanical extract treatments while no growth and sporulation of pathogen was observed at 10% concentration except *Aloe vera* wherein poor growth and sporulation were observed.

**Table 3b**  
In vitro evaluation effect of 10% herbal extract over growth and sporulation of pathogen, *Rhizopus stolonifer*

| Sr. no. | Herbal extract treatment at 5% concentration | Mean colony diameter (mm) | Per cent inhibition over control | Growth | Sporulation |
|---------|--|---------------------------|----------------------------------|--------|-------------|
| 1       | Garlic                                       | 0.00                      | 100.00                           | -      | -           |
| 2       | Onion  | 0.00                      | 100.00                           | -      | -           |
| 3       | Aloevera                                     | 13.33                     | 85.18                            | ++     | ++          |
| 4       | Kanher                                       | 0.00                      | 100.00                           | -      | -           |
| 5       | Nilgiri                                      | 0.00                      | 100.00                           | -      | -           |
| 6       | Sadafuli                                     | 0.00                      | 100.00                           | -      | -           |
| 7       | Zandu  | 0.00                      | 100.00                           | -      | -           |
| 8       | Neem   | 0.00                      | 100.00                           | -      | -           |
| 9       | Babul  | 0.00                      | 100.00                           | -      | -           |
| 10      | Tulsi  | 0.00                      | 100.00                           | -      | -           |
| 11      | Control                                      | 90.00                     | 00.00                            | -      | -           |
|         | SE (±)                                       | 0.50                      |                                  |        |             |
|         | CD@1%  | 2.00                      |                                  |        |             |

- = No growth, ++ = Poor, +++ = Medium, ++++ = Good

Thus, the results indicated that 10% concentration of extracts of all the botanicals were effective against the post harvest pathogen *Rhizopus stolonifer* than at 5% concentration. Whereas herbal extract of *Aloe vera* was not effective against the pathogen.

The results are in consonance with those reported by Narain and Satapathy (1977) who studied the antifungal characteristics of leaf, flower, stem and root extract of two varieties of *Vinca rosea* against *Fusarium oxysporum*, *Colletotrichum spp.* and *Aspergillus niger*. The extracts inhibited spore germination, sporulation and mycelial growth of test fungi. Jat and Goyal (2007) reported that the *Aspergillus* rot and blue mould rot caused by

*Aspergillus niger* and *Penicillium spp.* respectively was controlled by *Azadirachta indica* leaf extract (5%) followed by carbendazim (0.1%). Kale (2007) and Kamble (2008) also observed effectiveness of *Allium sativum* against *Colletotrichum gloeosporioides* in sweet orange. Antifungal activity of *Azadirachta indica* was reported by Gohil and Vala (1996), Asha and Thamaria (1977) and Lal *et al.* (1998) against *Alternaria alternata*. Where as *Allium sativum* and *Vinca rosea* were reported fungitoxic to *Colletotrichum gloeosporioides* by Ahmed and Agnihotri (1972) and Narain and Satapathy (1977).

## CONCLUSION

In the study of effect of herbal extract over growth and sporulation of all 3 pathogens, It was observed that amongo 5 and 10% concentration, all herbal extract were effective against all three pathogens at 10% concentration except *Aloevera*.

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