*Indian J. Hort. 67(3), September 2010: 306-310*

# Bioefficacy of indigenous plant extracts in controlling post-harvest stem-end rot (*Botryodiplodia theobromae*) of Kinnow fruits

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## ABSTRACT

**The Kinnow fruits are prone to attack by a variety of microorganisms that causes post-harvest fruit rots. Among these, stem-end rot incited by *Botryodiplodia theobromae* Pat. is most destructive and causes more than 20% of the total fruit rot in Sriganganagar (Rajasthan). Aqueous extract of nine indigenous medicinal and aromatic plants were evaluated against *B. theobromae* under *in vitro* and further explored their bioefficacy to manage stem-end rot of Kinnow fruits. 15% concentration of *Allium sativum* inhibited 100% mycelial growth of the pathogen followed by *Curcuma longa* (77.35%) and *Lawsonia inermis* (73.64%). The fruits treated with extract of *A. sativum* at 15% concentration showed significantly less incidence of rot to rest of the extracts tested in both pre- (82.92%) and post-inoculation (77.47%) treatments. The rot incidence occurred at 8th day of inoculation was significantly higher to that at 4th day of inoculation.**

**Key words:** Kinnow, post-harvest stem-end rot, *Botryodiplodia theobromae*, plant extracts.

## INTRODUCTION

Kinnow is a hybrid variety of mandarin group of citrus. In North-West India, the cultivation of Kinnow has gained importance, because of its precocious bearing and high yield potential (Gill *et al*., 3). The Kinnow fruits are prone to attack by a variety of microorganisms that causes post-harvest fruit decay (Bamba, 2). The conservative estimation on post-harvest losses (spoilage) of Kinnow fruits has been observed around 25-30% (Singh *et al*., 12). Stem-end rot caused by *Botryodiplodia theobromae* is a serious post-harvest disease of Kinnow fruits in Sriganganagar (Rajasthan), causing 20% of total fruit loss (Sharma, 10). The rot started as a slight discolouration around the stem-end but soon the infected part turn brown to black. It develops unevenly at the rind, forming finger like projections of brown to black discolouration at the lesion margins between the segments (Sharma, 10).

Although satisfactory control of this malady by using various chemicals has been documented in the literature (Godara, 4) but in addition to higher cost, the continuous use of these agro-chemicals for controlling the fruit decay may pose several problems like environmental pollution, development of resistance among the population of pathogen and hazardous to human health (Unnikrishnan and Nath, 14). Therefore, as an alternative to fungicide application it is desirable to exploit other cheaper, ecologically safer and effective control methods to manage the disease. Several plant extracts are reported to suppress many plant pathogens effectively (Sharma *et al*., 11). The plant extracts have no residual effect, are easily biodegradable and stimulate host metabolism. A new generation of different kinds of such pesticides would be economical and safer to use. Under these circumstances, management of post-harvest rots of Kinnow fruits with the help of these plant extracts appears to be an exhaustive approach.

Present investigation was undertaken to evaluate different indigenous medicinal and aromatic plants extracts against *B. theobromae* Pat. and further explored their bioefficacy to manage the incited post-harvest stem-end rot incidence in Kinnow fruits.

## MATERIALS AND METHODS

Extracts of the locally available nine plants having medicinal and aromatic values *viz.,* leaves of *Withania somnifera* (Ashwagandha), *Aloe barbedensis* (Guarpatha), *Lawsonia inermis* (Henna), *Azadirachta indica* (Neem), *Eucalyptus amygdalina* (Eucalyptus) and *Ocimum sanctum* (Tulsi), cloves of *Allium sativum* (Garlic), bulbs of *A. cepa* (Onion) and rhizomes of *Curcuma longa* (Turmeric) were selected to evaluate their fungitoxic activities against the *B. theobromae* and test their bioefficacy to manage the incited post-harvest stem-end rot of Kinnow fruits.

For extraction, parts of selected plants were collected and thoroughly washed with running tap water, rinsed with sterile distilled water and then air-dried. Weighed plant material was macerated with

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equal volume of sterile distilled water (1:1 w/v) in a warring blender. The material was homogenized for 5 min. and then filtered through double layered muslin cloth. The filtrate was centrifuged at 5000 rpm for 20 min. The supernatant was collected and filtered through Whatman filter paper No. 42 and autoclaved at 1.045 kg $cm^{-2}$ (15 psi) for 20 min. This filtrate was considered as standard plant extract solution of 100%.

The efficacy of plant extracts at different concentrations (5, 10 & 15 %) against the *B. theobromae* was tested *in vitro* by using 'Poisoned Food Technique' (Nene and Thapliyal, 7). The extracts were mixed separately with molten PDA to get concentrations of 5, 10 & 15% of the extracts and poured aseptically into sterile petri plates. Mycelial discs (5 mm dia.) of 7-day-old culture of *B. theobromae* were seeded in the center of the individual plate. Suitable control was maintained where the culture disc was grown under the same conditions on PDA without adulteration. Each treatment was replicated thrice. The petri plates were incubated at 25 ± 1°C temperature in BOD chamber. Observations were taken when the control plates were fully covered with mycelial growth of the *B. theobromae*. The per cent inhibition in mycelial growth of the *B. theobromae* compared to control was calculated as: I = C – T/C x 100 where, I = Inhibition (%), C = Colony diameter in control (mm), T = Colony diameter in treatment (mm). The data were statistically analysed using a factorial randomized block design.

Out of nine plant extracts tested against the mycelia growth of the pathogen, five most effective were selected for further testing their bioefficacy to manage the stem-end rot. The fruits were surface sterilized by dipping in 0.1% mercuric chloride solution for 1 min. followed by three washing with sterile distilled water and made injury by 'pin-prick method' up to the depth of 2 mm, at 5 places/fruit. The fruits were inoculated by dipping in spore suspension ($10^6$ spores $ml^{-1}$) of the *B. theobromae* for 2 min. Each plant extract was dissolved in sterile distilled water so as to get desired concentration (15%) and used for dip treatment (Thompson, 13) both as pre- as well as post-inoculation treatments. In case of pre-inoculation treatment, the fruits were first dipped in the test plant extract for 5 min., air-dried for 15 min. and then inoculated, while in the post-inoculation treatment, the fruits were first inoculated and then treated with plant extract. Parallel controls with fruits dipped in sterile distilled water were run simultaneously. The interval between pathogen inoculation and plant extract treatment or *vice-versa* was of 12 h. Each treatment was replicated thrice and having seven fruits in each replication. The experimental laid out in was factorial randomized block design.

The inoculated fruits were enclosed separately in pre-sterilized polythene bags (100 gauge) which perforated and partially sealed with paper pins and incubated at 25 ± 1°C temperature and 90-100% RH. The numbers of wounds showed rotting were recorded on 4th and 8th day of inoculation and fruit rot incidence (%) was calculated. The rot reduction index (RRI) in treated fruits compared to untreated control was calculated as: rot reduction (%) = [(% rot incidence in control - % rot incidence in treatment)/% rot incidence in control] × 100.

## RESULTS AND DISCUSSION

All the plant extracts used in present study, showed fungitoxicity against the *B. theobromae* under *in vitro* conditions. The mycelial growth of the pathogen was found to be reduced significantly even at 5% concentration compared to check (Table 1; Fig. 1). *A. sativum* inhibited 100% growth at 15% concentration. *C. longa*, *L. inermis*, *E. amygdalina* and *O. sanctum* were next in order of efficacy against the pathogen, exhibited 77.35, 73.64, 40.68 and 36.98% reduction in growth respectively. By increasing concentration of extracts, a progressive decrease in growth was observed, thereby, maximum growth inhibition was obtained at 15% concentration of the extracts. At 5, 10, and 15% concentrations of the extracts, the inhibition in the mycelial growth of the pathogen was recorded as 26.91, 41.09 and 53.61%, respectively which differed to each other significantly.

The effectiveness of plant extracts against the pathogen might be due to their active inhibitory substances such as alkaloides, phenols and phytoalexins which are known for their antifungal activities (Khilare and Gargawane, 5). Similar to present findings, antimicrobial potency of different plant extracts have been established earlier by Patra *et al*. (9). Yadav and Majumdar (15) has also been reported the efficacy of *E. amygdalina* against the *B. theobromae*. The effectiveness of *E. amygdalina* might be attributed to the presence of volatile compound 'Cineole' (Arya, 1).

In general, all plant extracts which tested, retarded the incidence of rot in treated fruits significantly in comparison to untreated control fruits. Rotting of fruits increased with increasing period of incubation. In pre-inoculation treatment, minimum incidence (11.67%) of rot was recorded in fruits which treated with extract of *A. sativum* significantly followed by 19.53, 23.33 and 32.86% rot incidence occurred in fruits dipped in aqueous solution of *C. longa, L. inermis* and *E. amygdalina* respectively. All extracts proved superior over the control where 68.34% rot incidence was observed. 26.51 and 40.16% rot incidence was noted at 4th and 8th day of inoculation, respectively which have significant difference (Table 2).

**Table 1.** Evaluation of plant extracts against *Botryodiplodia theobromae* under *in vitro* conditions.

| Plant extract | Common name | Inhibition of mycelial growth (%) | | | |
|---|---|---|---|---|---|
| | | 5% | 10% | 15% | Mean |
| *Withania somnifera* | Ashwagandha | 7.59 (15.98)* | 23.15 (28.75) | 37.96 (38.04) | 22.90 (27.59) |
| *Allium sativum* | Garlic | 69.07 (56.21) | 86.85 (68.74) | 100.00 (90.00) | 85.31 (71.65) |
| *Aloe barbedensis* | Guarpatha | 12.04 (20.29) | 27.78 (31.81) | 42.59 (40.74) | 27.47 (30.95) |
| *Lawsonia inermis* | Henna | 57.41 (49.26) | 76.67 (61.12) | 86.85 (68.74) | 73.64 (59.71) |
| *Azadirachta indica* | Neem | 4.26 (11.84) | 12.96 (21.10) | 27.78 (31.81) | 15.00 (21.58) |
| *Allium cepa* | Onion | 11.30 (19.62) | 26.67 (31.09) | 40.19 (39.34) | 26.05 (30.02) |
| *Eucalyptus amygdalina* | Eucalyptus | 23.52 (29.00) | 40.74 (39.66) | 57.78 (49.48) | 40.68 (39.38) |
| *Curcuma longa* | Turmeric | 63.52 (52.84) | 78.33 (62.26) | 90.19 (71.75) | 77.35 (62.28) |
| *Ocimum sanctum* | Tulsi | 20.37 (26.83) | 37.78 (37.93) | 52.78 (46.59) | 36.98 (37.12) |
| Control | - | 0.00 (0.57) | 0.00 (0.57) | 0.00 (0.57) | 0.00 (0.57) |
| Mean | | 26.91 (28.24) | 41.09 (38.30) | 53.61 (47.71) | |

*Figures in parenthesis are angular transformed values.

CD (*P = 0.05*) Plant extract = 0.58; Concentration = 0.32; Plant extract × Concentration = 1.00; CV (%) = 1.61

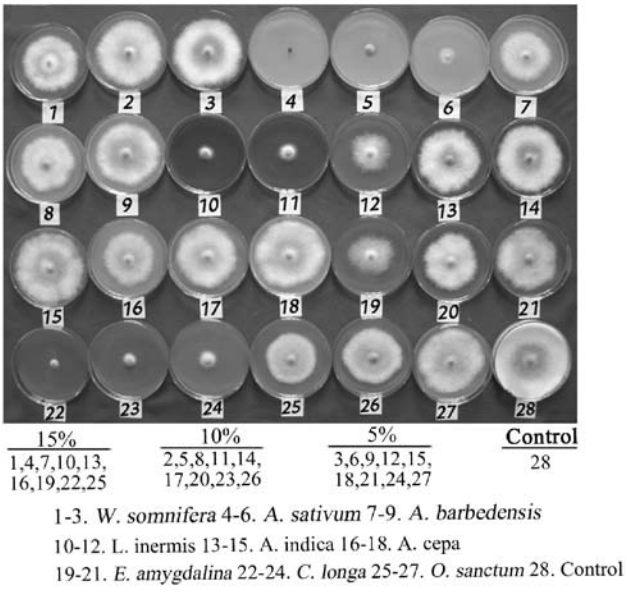


**Fig. 1.** Inhibition of mycelial growht of *B. theobromae* by plant extracts at different concentrations.

In post-inoculation treatment, extract of *A. sativum* proved again significantly superior over all other extracts used in controlling the rot development allowed just 15.72% rot incidence in treated fruits (Table 2). Extract of *C. longa* was next in order of efficacy against the rot allowed 22.62% rot incidence in treated fruits followed by *L. inermis* (27.62%) and *E. amygdalina* (42.15%), which was found significantly less compared to control (69.77%). The rot incidence at 8$^{th}$ day of inoculation (46.99%) was significantly higher to the incidence occurred at 4$^{th}$ day of inoculation (30.40%).

These findings are corroborated with those of Obagwn and Korsten (8) who achieved complete control of rotting in Valencia orange fruits with aqueous solution of *A. sativum*. The effectiveness of *A. sativum* might be due to the presence of sulphur compound 'Allicin' in them (Arya, 1). Mehta and Mehta (6) and Sharma *et al*. (11) have also been reported the efficiency of plant extracts in management of post-harvest fruit rotting. Moreover, it was observed that the plant extracts were more efficacious when used as pre-inoculation compared to post-inoculation treatment

**Table 2.** Efficacy of plant extracts against stem-end rot in post-harvest treated Kinnow fruits.

| Plant extract | Rot incidence in pre-inoculation (%) | | | | Rot incidence in post-inoculation (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 4th day | 8th day | Mean | RRI | 4th day | 8th day | Mean | RRI |
| *Allium sativum* | 9.53 (17.93)* | 13.81 (21.77) | 11.67 (19.85) | 82.92 | 12.86 (20.99) | 18.57 (25.52) | 15.72 (23.26) | 77.47 |
| *Lawsonia inermis* | 18.09 (25.16) | 28.57 (32.30) | 23.33 (28.73) | 65.86 | 21.43 (27.57) | 33.81 (35.55) | 27.62 (31.56) | 60.41 |
| *Eucalyptus amygdalina* | 26.67 (31.07) | 39.05 (38.66) | 32.86 (34.87) | 51.92 | 33.81 (35.54) | 50.48 (45.27) | 42.15 (40.41) | 39.59 |
| *Curcuma longa* | 15.72 (23.34) | 23.33 (28.85) | 19.53 (26.10) | 71.42 | 17.14 (24.44) | 28.10 (31.99) | 22.62 (28.22) | 67.58 |
| *Ocimum sanctum* | 35.24 (36.41) | 53.34 (46.91) | 44.29 (41.66) | 35.19 | 41.91 (40.33) | 66.67 (54.76) | 54.29 (47.55) | 22.19 |
| Control | 53.82 (47.19) | 82.86 (65.96) | 68.34 (56.58) | 0.00 | 55.24 (48.01) | 84.29 (67.06) | 69.77 (57.54) | 0.00 |
| Mean | 26.51 (30.18) | 40.16 (39.08) | | | 30.40 (32.81) | 46.99 (43.36) | | |

Figures in parenthesis are angular transformed values, RRI = Rot Reduction Index

| | | | | |
|---|---|---|---|---|
| CD (*P = 0.05*); Plant extract | = | 1.71 | = | 1.40 |
| Day | = | 0.99 | = | 0.81 |
| Plant extract × Day | = | 2.42 | = | 1.98 |
| CV (%) | = | 4.13 | = | 3.07 |

which might be due to lack of time for establishment and multiplication of pathogen of the rot.

To avoid the possible risk of development of resistant strains of *B. theobromae* against the chemicals, the extract of *A. sativum* @ 15% as pre-inoculation treatment may therefore be recommended as an alternative to chemicals for effective, cheaper and eco-friendly management of post-harvest stem-end rot of Kinnow fruits. Further studies are needed to isolate and characterize the antifungal substances present in these extracts for control of fruit rots with more efficient manner at large scale.

## ACKNOWLEDGEMENT

The authors are grateful to the Director Research, Rajasthan Agricultural University, Bikaner for providing facilities.

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Received: January, 2010; Revised: May, 2010;
Accepted : June, 2010