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Dhanshree Bachkar
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India

Sanjay Kolase
Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Mayur Bhujbal
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India

Ravindra Patil
Department of Horticulture, College of Agriculture, Dhule, Maharashtra, India

Mahadev Khatal
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India

Navnath Daingade
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India

Sadanand Palaskar
Department of Entomology, College of Agriculture, Dhule, Maharashtra, India

Corresponding Author:
Dhanshree Bachkar
Department of Plant Pathology, College of Agriculture, Dhule, Maharashtra, India

In vitro efficacy of different botanicals against *Fusarium incarnatum* causing fruit rot of papaya (*Carica papaya* L.)

Dhanshree Bachkar, Sanjay Kolase, Mayur Bhujbal, Ravindra Patil, Mahadev Khatal, Navnath Daingade and Sadanand Palaskar

Abstract
Papaya (*Carica papaya* L.) is an important and most widely grown fruit crop of both tropics and subtropics of the world, belonging to the family Caricaceae and ranks third in importance among fruits. Papaya fruits lose their market value due to damage caused by many fungi. These fungi by their prolific growth, deteriorates fruit quality. Among these, fruit rot caused by *Fusarium incarnatum* adversely affects the fruit quality, quantity and ultimately reduces the market value. The fruit rot of papaya causes enormous yield losses, often in field and markets. Detailed investigations on various aspects were carried out in the present study during 2019-20. The papaya fruits showing typical characteristic symptoms of fruit rot were collected from Pachkandil vegetable market, Dhule. Infected fruits exhibited water-soaked spots at stem-end portion and also showed softening and mummification of fruits. In severe cases, rotten fruit showed white creamy growth of the pathogen. The pathogen was isolated by standard tissue isolation method and purified by single spore technique. Pathogenicity of fungus was proved by following Koch's postulates. The fruit rot causal fungus was got identified by AGHARKAR RESEARCH INSTITUTE (An Autonomous body under the Department of Science and Technology, Govt. of India, G. G. Agarkar Road, Pune – 411 004) as *Fusarium* sp. aff. *F. semitectum* Berk & Ravenel (Current name- *Fusarium incarnatum* (Desm.) Sacc.) (ID.NO.3/426/2019/MYC/1135).
In vitro evaluation of the botanicals revealed that garlic clove / bulb extract @ 5% showed highest mycelial growth inhibition of the test pathogen and it was least with neem leaf extract @ 5%. Neem seed kernel extract @ 10% showed highest mycelial growth inhibition, followed by garlic clove extract. Tulsi @ 10 per cent, neem leaf @ 5 and @ 10 per cent were least effective.

Keywords: Papaya, *Carica papaya* L., *Fusarium incarnatum*, botanicals, inhibition

Introduction
Papaya (*Carica papaya* L.) is an important and most widely grown fruit crop of both tropics and subtropics of the world, belonging to the family Caricaceae and ranks third in importance among fruits. *Carica* is the largest of the four genera with 48 species, among which *Carica papaya* L. is most important and cultivated all over the world (Badillo, 1971 and Waller, 1992) [3, 12]. The popularity of papaya fruit has made it ubiquitous in tropical and subtropical regions of the world. Papaya is the native of tropical America (Singh, 1990) [9].
Papaya cultivation has became increasingly popular since, mid-nineteenth century because of its varied climatic tolerance and high nutritive values. The major papaya growing continents are Asia, South America, North Central America and Africa. About 65 per cent of the world's production is from South America. Another 35 per cent is from North Central America and Africa (Tasiwal and Benagi, 2008) [10]. In India, the papaya is grown for table purpose, papain and pectin extraction and concentrated in the state of Kerala, Orissa, West Bengal, Karnataka, Assam and Gujarat. In India, 1,38,400 ha area is covered under papaya with a production of 59,88,800 metric ton with an average productivity of 43.3 metric ton per ha during 2017-18. In Maharashtra, 10,280 ha area is covered under papaya with a production of 4,08,000 metric ton with an average productivity of 39.71 metric ton per during 2017-18 (Anonymous, 2018) [2].
The harvested papaya fruits always succumb to the infection by various pathogens causing fruit rot. Post-harvest diseases of papaya caused by fungi are responsible for causing losses to the tune of 45 per cent of their market value (Abeywickrama *et al.*, 2012) [1]. Fruits are living entities and are highly perishable commodities that are affected by number of factors leading to be post-harvest spoilage and hence, post-harvest losses are major one. Post-harvest diseases of fresh fruits are traditionally being controlled by synthetic chemical fungicides (Eckert and Ogawa, 1985) [4]. Papaya fruits are highly perishable in nature and it is very difficult to store for longer period, therefore, it needs immediate marketing and utilization.

Any physical damage like bruising or wound scratches to fruits makes them vulnerable / susceptible to many pathogens, resulting in heavy post-harvest losses. Reducing post-harvest losses in papaya fruit is an imperative aspect of research to find out the important pathogens attacking fruits during transit and storage, so as to advise appropriate management strategies and consequently to minimize post-harvest fruit losses in papaya. Considering these issues, present studies were undertaken on fruit rot of papaya,

Material and Methods

Collection, isolation, purification, identification and pathogenicity of the pathogen

Papaya fruits showing typical symptoms of fruit rot were collected from the Pachkandil vegetable market, Dhule, brought to the laboratory and subjected to tissue isolation of the pathogen.

Diseased papaya fruit tissues along with healthy tissues were cut and surface sterilized by dipping in 0.1 per cent mercuric chloride solution for one minute, followed by three successive washings with distilled sterile water. These pieces were aseptically placed on solidified Potato Dextrose Agar (PDA) medium (20 ml) in Petri plates and incubated at 28 ^{0}C in BOD incubator, for seven days. The fungus was subcultured, purified by single spore isolation and maintained on PDA slant tubes.

Identification of the pathogen was carried out by studying the cultural and morphological characters. Microphotographs of mycelium and spore structure were taken with the help of digital camera. The pure culture was sent to Agharkar Research Institute (ARI), Pune for identification. They identified the pathogen as *Fusarium* sp. aff. *F. semitectum* Berk and Ravenel (Current Name - *Fusarium incarnatum* (Desm.) Sacc.), solely based on morphological characters.

For pathogenicity test conidial suspension was prepared (4 x 10^6 cfu/ml) by adding sterile distilled water to the inoculum. The fruits were inoculated with syringe by inoculating the conidial suspension, prepared from seven days old culture in sterile distilled water and incubated in moisture chamber to ensure successful infection. Observations were recorded for the appearance and development of the symptoms. After symptom development, reisolation was done from the artificially infected fruits and compared it with original culture for confirmation.

In vitro efficacy of botanicals

Aqueous phytoextracts of five plant species were evaluated *in vitro* (each @ 5 and 10 per cent) against the test pathogen, by applying Poison Food Technique (Nene and Thapliyal, 1982). Fresh plant material collected was first washed in running tap water and then in distilled water. Hundred grams of fresh sample was chopped and crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through double layered muslin cloth and the filtrate thus obtained (100%) was used as stock solution.

Five ml and 10 ml of stock solution was mixed with 95 and 90 ml of sterilized molten PDA media, respectively and mixed thoroughly, so as to get 5 and 10 per cent concentration. Twenty ml of poisoned medium was poured into each of the 90 mm sterile petri plates. Each plate was seeded with actively growing culture disc (5mm) of the test pathogen by placing at centre of each agar plate. Untreated control was maintained by inoculating PDA plates with culture disc of the test pathogen. Then such plates were incubated at 27 ± 1 ºC temperature for seven days. Observations on radial mycelial growth were recorded when untreated control plates were fully covered with the growth of test pathogen. Per cent mycelial growth inhibition with the test phytoextracts was calculated by using the formula suggested by Vincent (1947) [11].

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

Where,
I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment

Experimental Details, as below

1. Design - CRD (Complete Randomized Design)
2. Replications – 3
3. Treatment - Botanicals – 6 (@ 5 and 10%)

Table 1: Treatment Details

Tr. No.	Treatments	Common Name	Plant part used	Family
T_1	*Allium sativum*	Garlic	Clove	Amaryllidaceae
T_2	*Azadirachta indica*	Neem	Leaves	Meliaceae
T_3	*Ocimum tenuiflorum*	Tulsi	Leaves	Lamiaceae
T_4	*Zingiber officinale*	Ginger	Rhizomes	Zingiberaceae
T_5	*Azadirachta indica*	Neem	Kernel	Meliaceae
T_6	Control (Untreated)	-	-	-

All numerical data was statistically analyzed by using the appropriate statistical methods (Rangaswami, 2006) [7], to assess statistical significance of the treatments.

Results and Discussion

In vitro efficacy of different botanicals against *Fusarium incarnatum*

The results (Table-1, Fig.-1, 2 and Plate-1) revealed that the test aqueous phytoextracts significantly inhibited mycelial growth of *F. incarnatum*, over untreated control. Garlic @ 5% resulted with significantly highest mycelial inhibition (51.52%) and lowest mycelial growth (42.66 mm) and moderate sporulation (++), followed by Tulsi (17.80%, 72.33 mm, good sporulation). Rest of the test phytoextracts were least effective against the test pathogen.

Table 1: *In vitro* efficacy of botanicals @ 5% against *F. incarnatum*

Tr. No.	Botanicals	Concentration (%)	Mean colony Diameter (mm)*	Sporulation	Per cent Inhibition (%)
T_1	Garlic (*Allium sativum*)	5	42.66	++	51.52
T_2	Neem leaf (*Azadirachta indica*)	5	85.66	+++	2.65
T_3	Tulsi (*Ocimum tenuiflorum)*	5	72.33	+++	17.80
T_4	Ginger (*Zingiber officinale*)	5	73.66	+++	16.29
T_5	Neem seed kernel extract (*Azadirachta indica*)	5	81.33	+++	7.57
T_6	Control (Untreated)	-	88	+++	-
	S.E. ±	-	0.69	-	-
	CD at 5%	-	2.16	-	-

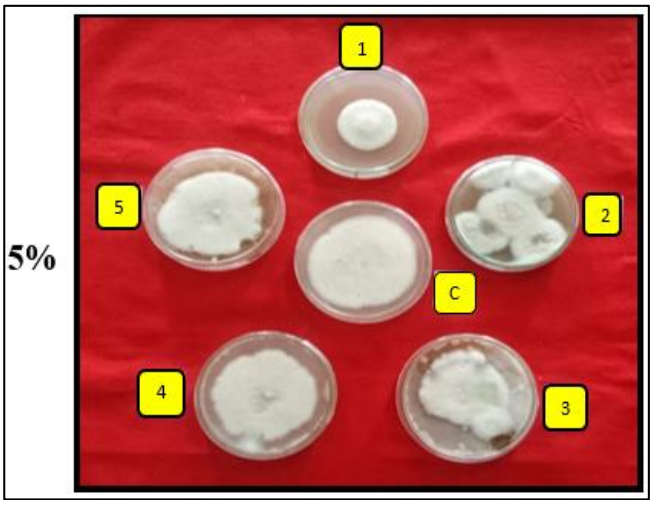
* = Average of three replications
+++ : Good sporulation
++ : Moderate sporulation
+ : Scanty sporulation
- : No sporulation

The results (Table-2) revealed that among the phytoextracts tested @ 10%, Neem seed kernel extract resulted with significantly highest mycelial growth inhibition (83.34%), lowest colony growth (14.66 mm) and no sporulation, followed by Garlic (58.71%) with colony growth (36.33 mm) and scanty sporulation and Ginger (24.25%) with colony growth (66.66 mm) and good sporulation. Rest of the test phytoextracts were least effective.

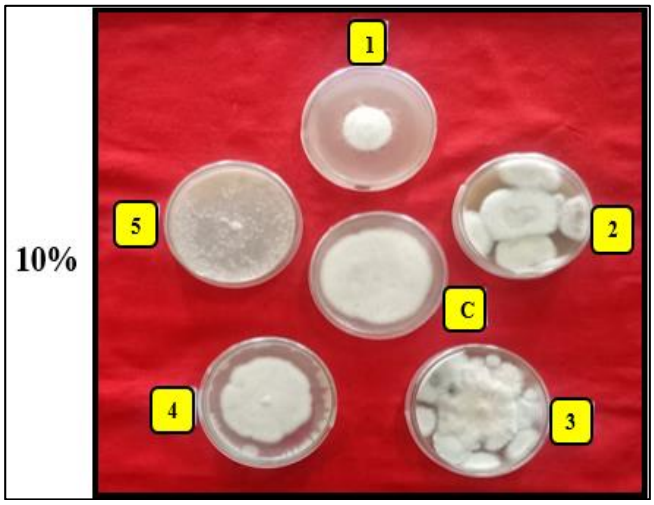
These results are on the same line of Riberio and Bendedo (1999) [8] who reported peppermint and garlic extract inhibited mycelial growth in the range of 5.3 to 67.6 per cent; however, it had no effect on sporulation of fungus of papaya fruit rot. Patel and Joshi (2001) [6] reported tulsi leaf extract as ineffective in inhibiting the mycelial growth of fungus.

Table 2: *In vitro* efficacy of botanicals @ 10% against *F. incarnatum*.

Tr. No.	Botanicals	Concentration (%)	Mean colony Diameter (mm)*	Sporulation	Per cent Inhibition (%)
T_1	Garlic (*Allium sativum*)	10	36.33	+	58.71
T_2	Neem leaf (*Azadirachta indica*)	10	87	+++	1.13
T_3	Tulsi (*Ocimum tenuiflorum*	10	87	+++	1.13
T_4	Ginger (*Zingiber officinale*)	10	66.66	+++	24.25
T_5	Neem seed kernel extract (*Azadirachta indica*)	10	14.66	-	83.34
T_6	Control (Untreated)	-	88	+++	-
	S.E. ±		0.57		
	CD at 5%		1.79		



a. Per cent inhibition of mycelial growth of *Fusarium incarnatum* @ 5%



b. Per cent inhibition of mycelial growth of *Fusarium incarnatum* @ 10%

Plate 1: *In vitro* efficacy of botanicals against *Fusarium incarnatum.* 1. Garlic 2. Neem Leaf 3. Tulsi 4. Ginger 5. Neem Seed Kernel Extract C. Untreated Control

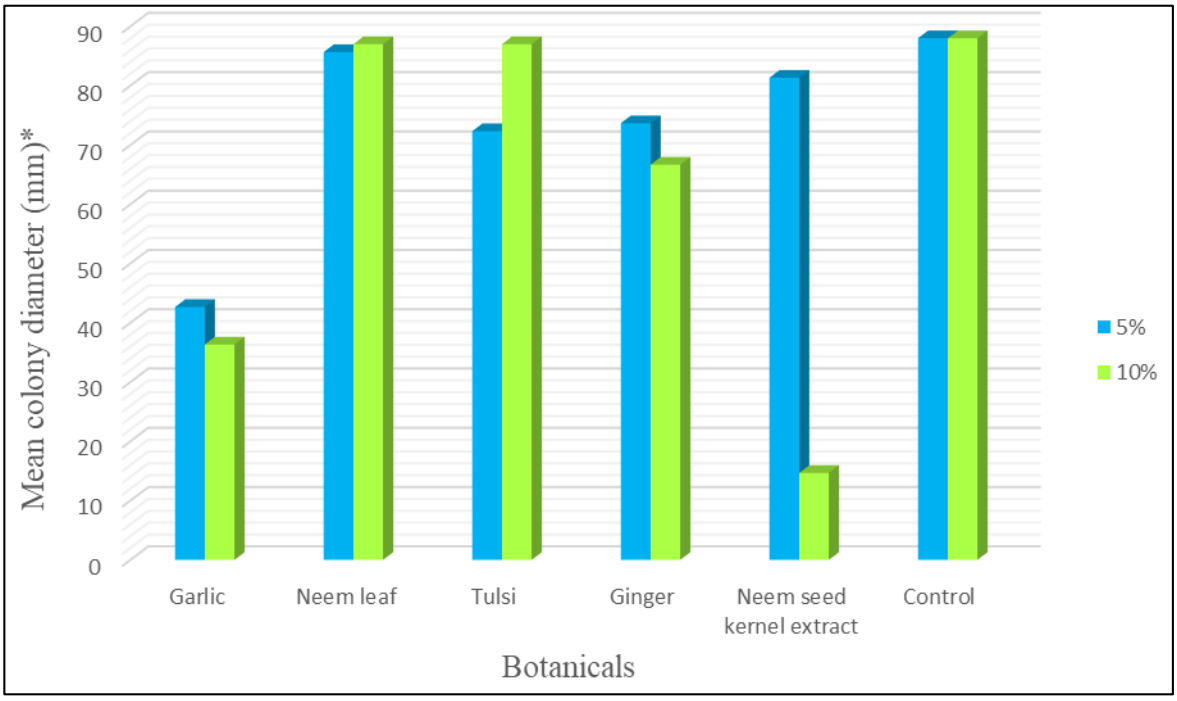


Fig 1: *In vitro* effect of botanicals on mycelial growth of *Fusarium incarnatum.*

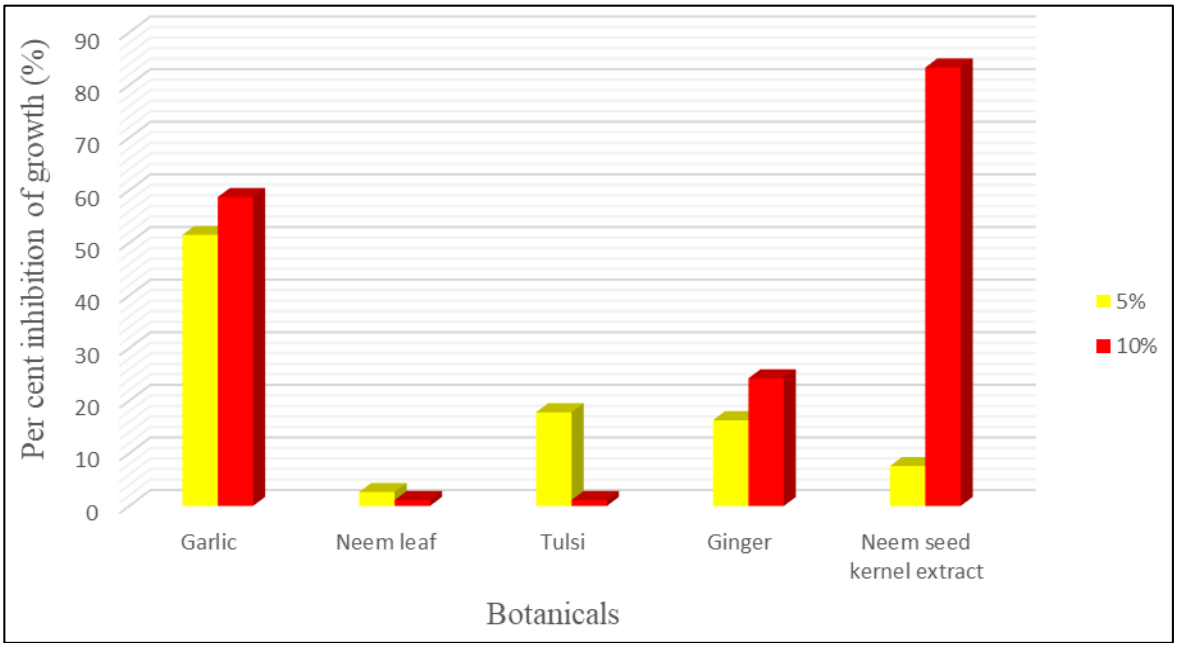


Fig 2: *In vitro* effect of botanicals on mycelial growth inhibition of *Fusarium incarnatum*

Conclusion

Hence, from ongoing results and discussion, it is concluded that *in vitro* testing of botanicals (@ 5 and 10%) against *Fusarium incarnatum* revealed that garlic @ 5% resulted with highest mycelial growth inhibition (51.52%) and moderate sporulation and rest were least effective. At 10% concentration, Neem seed kernel extract resulted with significantly highest mycelial growth inhibition (83.34%) without sporulation, followed by garlic (58.71%) with scanty sporulation and ginger (24.25%) with good sporulation.

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