

Investigations on Leaf Blight Disease of Clove Incited by *Cylindrocladiumquinqueseptatum* Boedijn & Reitsma

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Abstract Clove (*Syzygium aromaticum*) (L) Merr. & Perry is one of the tree spices noted for its flavor and medicinal values. It is an aromatic plant and imparts warming qualities when it is used as culinary spice. A severe leaf blight incidence was observed on the clove trees at the Horticultural Farm, College of Agriculture, Dapoli during the rainy season. The total severity of the disease resulted in rapid blighting and heavy defoliation. The causal organism of the disease was isolated and identified as *Cylindrocladiumquinqueseptatum*. The *in vitro* evaluation of different fungicides revealed that Carbendazim and Carbendazim + Mancozeb each at 0.1% concentration completely inhibited growth of the pathogen. While in the field trial on the clove trees, Carbendazim (0.1%) was the most effective in controlling the leaf blight disease.

Keywords: leaf blight, fungicides, host, pathogen, *Syzygium aromaticum*

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1. Introduction

Clove - *Syzygium aromaticum* (L.) Merr. & Perry (2n=16, 4n=32), is a tree spice belong to family Myrtaceae famous for its aroma and medicinal values. Clove was introduced to India around 1800 AD, by the East India Company in their spice garden at Courtallam, Tamil Nadu. The major producers of this spice today are Indonesia, Zanzibar and Madagascar [14]. Clove imparts warming qualities when it is used as a culinary spice. It has expectorant and antiemetic properties. It is also used as an anodyne and antiseptic in dentistry. It is carminative and stimulant and used against flatulence and dyspepsia. It is also a constituent of tooth paste and mouth washes. The clove oil possesses antiseptic and antibacterial properties and hence used in many pharmaceutical preparations. It is also extensively employed in perfumes and soaps [14].

Clove product may be considered in three major forms: clove buds are used as such or utilized in the preparation of ground spice, oleoresin or for the distillation of bud oil; stem oil and leaf oil. Stem and leaf oil are principally used as source of eugenol. In the 'Konkan' region of Maharashtra state there are no plantations of clove but some farmers have grown it as a kitchen garden tree and as a novelty crop. Clove trees suffer from leaf blight disease caused by *Cylindrocladiumquinqueseptatum* particularly during monsoon season.

2. Materials and Methods

The leaf blight of clove initiates as spots on the leaves gradually resulting in leaf blight followed by severe defoliation.

2.1. Isolation of the Causal Organism

The fresh samples of clove leaves showing typical symptoms of the disease were collected from the Horticulture Farm, College of Agriculture, Dapoli and brought to the laboratory in a clean polythene bag and were used for the isolation. The pathogen was isolated on potato dextrose agar medium. The diseased samples were washed with sterilized water to remove extraneous material. Small bits of affected portion along with the healthy tissue were cut from the peripheral margins of spots and surface sterilized in 0.1 per cent mercuric chloride solution for 1 minute followed by washing thrice in sterilized distilled water to remove traces of mercuric chloride. The pieces were then aseptically transferred in PDA. The inoculated Petri plates incubated at room temperature (27±1°C). Growth of the fungus was obtained in 5 to 6 days. Pure culture was maintained for further studies by periodical transfer on PDA slants.

2.2. Chemical Control *in Vitro*

Six fungicides viz., Tricyclazole, Zineb, Carbendazim + Mancozeb, Hexaconazole, Copper hydroxide and Carbendazim were tried against the pathogen by poisoned food technique (PFT) described in reference no. [8].

The weighed quantity of each fungicide/fungicidal combination was added separately to PDA medium in flasks and mixed thoroughly. The mycelial discs of 5 mm

diameter of 10 days old culture were transferred aseptically to the center of the solidified medium in Petri plates. The PDA plates without fungicide served as control. Three replications per treatment were maintained and the plates were incubated at room temperature ($27\pm 1^\circ\text{C}$). The observations for colony diameter for the fungus and sporulation were recorded until Petri plates in control were fully covered with mycelial growth.

The percent inhibition of the fungal growth was calculated by the following formula [10]:

$$x = \frac{y-z}{y} \times 100$$

Where

X= per cent inhibition

Y= Growth of fungus in control (cm)

Z= Growth of fungus in treatment (cm)

2.3. Chemical Control *in Vivo*

The disease was appeared in clove orchard of Department of Horticulture, College of Agriculture, Dapoli on the onset of monsoon. Spraying of fungicides was carried out on infected foliage in concentration specified in table no 1.

The experiment was carried out in Randomized block design with three replications and seven treatments in the months of June to September for two subsequent monsoons. The data recorded were statistically analyzed and the differences exhibited by the treatments were tested for their significance as per the methods suggested in reference no. [13].

Table 1. Treatment details

Treatment	Name of fungicide used	Active Ingredient	Concentration (%)
T ₁	Tricyclazole (Beam)	75 WP	0.1
T ₂	Zineb (Dithane Z 78)	78 WP	0.1
T ₃	Carbendazim + Mancozeb(Saaf)	12 + 63 WP	0.1
T ₄	Hexaconazole (Contaf)	5 EC	0.1
T ₅	Copper hydroxide	50 WP	0.1
T ₆	Carbendazim (Bavistin)	50 WP	0.1
T ₇	Control		

Methods of recording observations

Three branches per plant were tagged randomly for recording for blight incidence.

First observation: 20 days after 1st application, Second Observation: 20 days after second application, Third Observation: 20 days after the third application, Fourth observations: 20 days after the fourth application: Fifth observation 20 days after the fifth application.

Table 3. Effect of different fungicides on growth and sporulation of *C. quinqueseptatum*

Fungicides	Conc (%)	Inhibition over control (%)	Colony dia (cm)	Sporulation /cm ²
Tricyclazole (Beam)	0.1	61.62	03.30	Good
Zineb (Dithane Z 78)	0.1	46.51	04.60	Fair
Carbendazim + Mancozeb based powder	0.1	100.00	00.00	Nil
Hexaconazole (Contaf)	0.1	58.13	03.63	Excellent
Copper hydroxide	0.1	5.81	08.10	Poor
Carbendazim (Bavistin)	0.1	100.00	00.00	Nil
Control		-	08.60	Excellent
Each value represents Means of 3 replication			S.E.=±0.238 C.D. at 1%=0.999 C.D. at 5%=0.722	

Sporulation per cm². Excellent = 11 and above spores. Good = 7 to 10 spores. Fair = 4 to 6 spores. Poor = 1 to 3 spores. Nil = No spore.

Disease scoring

The disease intensity was recorded on the basis of healthy and infected leaves. Per cent disease incidence was calculated by the formula suggested in reference no. [12]

$$PDI = \frac{\text{No. of infected leaves}}{\text{Total no healthy and infected leaves assessed}} \times 100$$

2.4. Host Range

This study was undertaken to determine the ability of the test fungus to infect different plants species occurring around in the locality of clove tree. Each host was inoculated manually with the pathogen. Very light injuries were made to surface of selected leaf with sand paper (No. 40) by gently pressing which facilitate easy penetration by the fungus. The homogeneous spore suspension (>200 conidia per microscopic field at low power) prepared in sterile water was sprayed by sterilized atomizer on the surface of the leaves. After inoculation, plants were covered with clean polyethene sheets of appropriate size for maintaining high humid conditions. Adequate control plants were simultaneously maintained in each host plant category tested. The observations for disease development were recorded after a period of 10 days. The following plants were selected for the study:

Table 2. Hosts used for inocutaion

Common name	Botanical name
All spice	<i>Pimentadiocia</i>
Cashew	<i>Anacardiumoccidentale</i>
Citrus	<i>Citrus sinensis</i>
Crotons	<i>Croton sparsiflorus</i>
Eucalyptus	<i>Eucalyptus citrodora</i>
Guava	<i>Psidiumguajava</i>
Jamun	<i>Syzygiumcumini</i>
Netmeg	<i>Myristicafragrans</i>
Sapota	<i>Manilkaraachras</i>
Tapioca (cassava)	<i>Manihotesculanta</i>

3. Results and Discussion

3.1. In Vitro Evaluation of Different Fungicides

In vitro evaluation of different fungicides was carried out by employing PFT technique. The data obtained on the effect of various fungicides *in vitro* on the vegetative growth and sporulation of *C. quinqueseptatum* is presented in Table 3.

The data presented in Table 3 indicate that the mycelial growth as well as sporulation of *C. quinqueseptatum* was completely (100%) inhibited by Carbendazim and Carbendazim + Mancozeb. Both these treatments were at par with each other and superior to the rest of the treatments. Tricyclazole, Hexaconazole and Zineb were effective in restricting the mycelium but ineffective in controlling sporulation. Copper hydroxide was the least effective fungicide in terms of mycelial inhibition but it effectively hindered the sporulation of the pathogen. According to reference no [3] Carbendazim is most effective against *C. quinqueseptatum*. while reference no [7] suggests Carbendazim is effective in inhibiting conidial germination of *C. colhounii*. Reference no [6]

reported that Benomyl, copper oxy-chloride; Metalaxyl+ Mancozeb, Mancozeb and Propineb were highly effective in inhibiting conidial germination of *C. quinqueseptatum*. These results are in conformity with the present findings. The results of reference no [1] who reported that Zineb could effectively check the conidia germination of *C. colhounii*, are contradictory to the results of the present investigation.

3.2. In vivo Efficacy of Different Fungicides against the Fungus

All the fungicides used in PFT were also tested under field conditions. The results are presented in Table 2.

Table 4. Efficacy of different fungicides against the fungus

Fungicide	Conc%	Per cent of disease intensity	Per cent Disease Control (PDC)
Tricyclazole (Beam)	0.1	50.52 (45.07)	39.39
Zineb (Dithane Z 78)	0.1	49.36 (44.43)	40.78
Carbendazim + Mancozeb based powder	0.1	21.30 (27.48)	74.44
Hexaconazole (Contaf)	0.1	51.24 (45.69)	38.53
Copper hydroxide	0.1	56.23 (48.54)	32.54
Carbendazim (Bavistin)	0.1	18.20 (25.77)	78.16
Control		83.36 (65.94)	100
	S.E.	±0.532	
	C.D. at 5%	1.638	

It is revealed from the data presented in Table 4 that all the fungicides were effective in controlling the disease. Carbendazim alone was the best fungicide for controlling the disease under field conditions followed by carbendazim in combination with mancozeb. Among all the treatments, Copper hydroxide was the least effective fungicide.

Reference no [2] reported that leaf spot of *Magnolia grandiflora* caused by *Cylindrocladium* spp. was completely controlled by Dithane M 45 80 WP. Dithane M 45 is effective against seedling blight of eucalyptus caused by *Cylindrocladium* spp. [9] According to reference no [4] leaf spot and blight of *Syzygiumcumini* caused by *C. Quinqueseptatum* was effectively controlled by combination of Dithane M 45 (mancozeb) + bavistin (carbendazim). *C. floridanum* and *C. scoparium* causing leaf spot and blight of *Shorearobusta* (sal) was effectively controlled by a mixture of Dithane M-45 (0.2%) and Bavistin (0.1%) [5]. these results are in conformity with present findings.

3.3. Host Range Studies

Table 5. Host range of *C. quinqueseptatum*

Host	Disease development
All spice (<i>Pimentadiocia</i>)	+
Cashew (<i>Anacardiumoccidentale</i>)	+
Citrus (<i>Citrus sinensis</i>)	+
Crotons (<i>Croton sparsiflorus</i>)	+
Eucalyptus (<i>Eucalyptus citrodora</i>)	+
Guava (<i>Psidiumguajava</i>)	+
Jamun (<i>Syzygiumcumini</i>)	+
Netmeg (<i>Myristicafragrans</i>)	+
Sapota (<i>Manilkaraachras</i>)	+
Tapioca (<i>Manihotesculanta</i>)	+

Disease development: + = Pathogenic ; - = Non pathogenic

In order to determine the host range of the test fungus, healthy ten different plants species were artificially inoculated with the test fungus. The inoculated plants then incubated. The data in respect of the host studies and development of symptoms are presented in Table 5.

It is clear from the data presented in Table 5 that all test plants were susceptible to *C. quinqueseptatum*. Characteristic leaf blight symptoms were observed on all the hosts within 8 to 10 days of inoculation. Reference no [11] also reported that Jamun (*Syzygiumcumini*) is a host of *C. quinqueseptatum*.

4. Conclusions

On the basis of results of the present study, it can be concluded that the mycelial growth as well as sporulation of *C. quinqueseptatum* were completely (100%) inhibited by Carbendazim and Carbendazim + Mancozeb. Tricyclazole, Hexaconazole and Zineb were effective in restricting the mycelium but ineffective in controlling sporulation. Copper hydroxide was the least effective fungicide under *in vitro* conditions while under field conditions Carbendazim alone was the best fungicide followed by carbendazim in combination with mancozeb for controlling the disease.

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Abbreviations

PFT: Poisoned Food Technique

PDA: Potato Dextrose Agar

PDI: Percent Disease Incidence

References

- [1] Andrade, A.M.; Lopes, C.A. and Reifschneider, F.J.B. "Effect of fungicides on inhibition of *Cylindrocladiumclavatum*, *in vitro* and *in vivo* on potato tuber." *Phytopathologia Brasileira*, 8 (3): 403-408, 1983.
- [2] Alferi, S.A. and Grahm, A.E. "Cylindrocladium leaf spot of Magnolia grandiflora in Florida." *Proc. Florida State Hort. Soc.*, 84: 354-356, 1971.
- [3] Anahosur, K.H.; Padaganoor, G.M. and Hedge, R.K. "Toxic effect of the culture filtrate of the *Cylindrocladiumquinqueseptatum* the causal organism of seedling blight of eucalyptus hybrid." *Pl. Dis. Repr.*, 16 (2): 84-85, 1976.
- [4] ArushiMehrotra and Mehrotra, M.D. "Leaf spot and blight, a new disease of *Syzygiumcumini* by two *Cylindrocladium* spp. From India." *Indian J. Forestry*, 234: 496-500, 2000.
- [5] ArushiMehrotra "Cylindrocladium leaf spot and blight, a new disease of *Shorearobusta*." *Indian J. Forestry*, 24 (1): 77-79, 2001.
- [6] Jayasinghe, C. K. and Wijesundera, R. L.C. "In vitro evaluation of fungicides against clove isolate of *Cylindrocladiumquinqueseptatum* Sri Lanka." *International J. Pest Management*, 41 (4): 291-223, 1995.
- [7] Nair, J.M and Jayasree, M.C. "Occurrence of *Cylindrocladiumcolhouii* Peeralay on eucalyptus in Kerala." *Curr. Sci.*, 55 (16): 799-800, 1986.
- [8] Nene, Y.L. and Thalpliyal, P.N. "Fungicides in plant disease control." 11th Edn. Oxford and IBH Publ., New Delhi, India: pp. 531-532, 1979.
- [9] Rattan, G.S. and Dhanda, R.S. "Leaf blight and seedling disease of eucalyptus caused by *Cylindrocladium* spp. in Punjab." *Ann. Biol. India*, 1 (2): 184-188, 1985.
- [10] Horsfall J. G. "Principles of Fungicidal Action" 1956. Chronicle Botanica publication (USA) 207 pp.
- [11] Sharma Y R and Nambian K K N. "Cylindrocladium rust of clove." *Pl. Dis. Rptr.* 62 (6): 562-564, 1978.
- [12] Wallen V R and Jackson H R. "Model for yield loss determination of bacterial blight of field beans utilizing derail infrared photography combined field plot studies." *Phytopath.* 65: 942-947, 1975.
- [13] Gomez, K. A. and A. A Gomez. "Statistical Procedures for Agricultural Research". 2nd ed. John Wiley and Sons, London, 1986, 680 pp.
- [14] K.L. Chadha. "Handbook of Horticulture" 1st edn. ICAR publications, 2012, 1069 pp.