Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **2 (3):** 338-343 (2014)

Research Article

In vitro Evaluation of Botanicals and Biocontrol Agents Against *Colletotrichum musae*, Causing Anthracnose of Banana

K. Venkata Ramesh*
Department of Plant Pathology, Sri Venkateswara Agricultural College, Tirupati
*Corresponding Author E-mail: kvrameshagri@gmail.com
Received: 17.04.2014 | Revised: 19.06.2014 | Accepted: 28.06.2014

ABSTRACT

Postharvest pathogen Colletotrichum musae was isolated from diseased banana fruits. In vitro evaluation of botanicals against Colletotrichum musae revealed that highest per cent inhibition (77.96 %) of mycelial growth of C. musae was recorded in neem leaf extract followed by Garlic bulb extract (60.03 %), tulsi leaf extract (57.00 %). Least inhibition (5.67 %) was recorded in tridax leaf extract. Highest inhibition of spore germination of C. musae was recorded in garlic bulb extract (65.71 %), followed by tulsi leaf extract (61.31 %), neem leaf extract (57.16 %). Among the biocontrol agents, Bacillus subtilis recorded highest inhibition (84.45 %) followed by Pseudomonas fluorescens (79.30 %) and Trichoderma. viride (77.02 %). Hence, these botanicals and biocontrol agents can be effectively used as alternatives to chemicals.

Keywords: *Pathogen, Tulsi, Neem, Biocontrol agents, Trichoderma*

INTRODUCTION

Postharvest losses of perishable crops in developing countries have been estimated in the range of 5-50 per cent or more of the harvest (Salunke and Desai, 1984). Postharvest losses up to 12-14 % have been reported from India (Madan and Ullasa, 1993). Anthracnose caused by *Colletotrichum musae* is one of the most important post harvest pathogen of banana. Postharvest diseases are traditionally controlled by chemicals but development of resistance in pathogens to fungicides and risk of fungicides towards public health and environment underlines the necessity to develop safe alternatives. There is an urgent need to develop novel and alternative postharvest disease management strategies. Non chemical management using botanicals and biocontrol agents provide an opportunity for addressing the fungicide residue problems in the management of postharvest diseases. The present *in vitro* investigations are carried out in UAS, Dharwad to find out suitable botanical and biological control agents against *Colletotrichum musae* isolated from banana fruits.

MATERIALS AND METHODS

Banana fruits infected by *Colletotrichum musae* showing typical symptoms (Plate 1) were collected from Dharwad market and banana orchards. Fungus was isolated by following standard tissue isolation method. Pathogenicity was proved by proving Koch's postulates.

Cite this article: *In vitro* Evaluation of Botanicals and Biocontrol Agents Against *Colletotrichum musae*, Causing Anthracnose of Banana, *Int. J. Pure App. Biosci.* **2(3):** 338-343 (2014).

A) *In vitro* evaluation of botanicals:

Antagonistic activity of the below mentioned botanicals was tested *in vitro.*

Sl. No.	Scientific name	Vernacular name	Family	Part used
1	*Allium sativum* L.	Garlic	Amaryllidaceae	Bulb
2	*Azardirachta indica* Juss.	Neem	Meliaceae	Leaves
3	*Clerodendron inermii* Gaertn.	Kashmir bouquet	Verbenaceae	Leaves
4	*Chromolaena odoratum* L.	Communist weed	Compositae	Leaves
5	*Lantana camara* L.	Lantana	Verbenaceae	Leaves
6	*Ocimum sanctum* L.	Tulsi	Lamiaceae	Leaves
7	*Parthenium hysterophorous* L.	Congress grass	Compositae	Leaves
8	*Tridax procumbens* L.	Tridax	Compositae	Leaves and flowers

Preparation of stock solution of Botanicals:

Fresh leaves/bulb of each botanicals plant was collected and washed first in tap water and then in distilled water. Then, 100 g of fresh sample was crushed in a mixer grinder by adding 100 ml sterile distilled water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Final filtrate thus obtained was used as stock solution.

i) Mycelial growth inhibition:

Antifungal activity of botanicals was tested using the poisoned food technique. Stock solutions of 5 ml and 10 ml were mixed with 95 and 90 ml of sterilized molten PDA medium respectively to get 5 and 10 per cent concentrations. Twenty ml of the poisoned medium was poured into each of the 90 mm sterilized petriplates. Each plate was seeded with 0.5 cm mycelial discs taken from the periphery of eight day old fungal culture and Per cent inhibition of mycelial growth over control was calculated when the growth of the fungus is full in control plate by using the formula given by Vincent (1927).

ii) Spore germination inhibition:

Effect of botanicals on spore germination of the test fungi was assessed by per cent inhibition of conidial germination. A single drop of the conidial suspension of the test organisms was added to the well of a series of cleaned cavity slides, to which a single drop of different botanicals (double the required concentrations) was also added to get the required concentrations of 5 and 10 per cent. The wells were immediately covered by using coverslips on the cavity slides and the periphery was smeared with Vaseline. Control was maintained with distilled water. The cavity slides were kept in the petriplates lined with moist blotting paper and were incubated at room temperature. Observations were made from ten microscopic fields from each slide. Per cent germination was calculated from the number of total conidia and germinated conidia in each microscopic field. Further, the percent inhibition of spore germination was calculated by using the formula given by Vincent (1927) for each botanical.

B) *In vitro* evaluation of biocontrol agents

Antagonistic activity of the below mentioned biocontrol agents was tested *in vitro.*

Sl. No.	Biocontrol agent
1	*Bacillus subtilis*
2	*Pseudomonas fluorescens*
3	*Trichoderma harzianum*
4	*T. pseudokoningi*
5	*T. reesei*
6	*T. virens*(isolate 1)
7	*T. virens*(isolate 2)
8	*T. viride*

From the actively growing cultures of both fungal bioagents and test pathogens, 0.5 cm fungal disc were transferred aseptically to petriplates containing PDA, simultaneously by leaving sufficient space in between two discs. In case of bacterial biocontrol agents, mycelial discs of the test fungus was kept at opposite ends and bacterium was streaked at the center. A pathogen disc alone placed at the center of the petriplate served as control. Colony diameter of both the test fungus and bioagents were measured when control plate is fully covered and per cent inhibition was calculated by using the formula given by Vincent (1927).

RESULTS AND DISCUSSION

A) *In vitro* evaluation of botanicals:

Effect of botanicals on *Colletotrichum musae* The data pertaining to the effect of plant extracts on mycelial growth and spore germination of *C. musae* are presented in Table 1.

i) Inhibition of mycelial growth:

Among the different treatrnents tested, highest per cent inhibition (77.96 %) of mycelial growth of *C. musae* was recorded in neem leaf extract followed by Garlic bulb extract (60.03 %), tulsi leaf extract (57.00 %). Least inhibition (5.67 %) was recorded in tridax leaf extract. Among the different concentrations, neem leaf extract at 5 per cent (71.12 %) and 10 per cent (84.80 %) were found to be superior over all other treatments. Garlic bulb extract at 10 per cent (68.87 %) and tulsi leaf extract at 10 per cent (65.50%) were on par with each other. Next best was garlic bulb extract at 5 per cent (52.21 %), followed by tulsi leaf extract at 5 per cent (48.50 %).

ii) Inhibition of spore germination:

Among the different botanicals tested highest (65.71 %) inhibition of spore germination of *C. musae* was recorded in garlic bulb extract followed by tulsi leaf extract (61.31 %), by neem leaf extract (57.16 %), lantana leaf extract (42.63 %) and chromolaena leaf extract (41.08 %). Among the different concentrations individually, highest inhibition (74.00 %) of spore germination was observed in garlic bulb extract at 10 per cent concentration, which was on par with tulsi leaf extract at 10 per cent (73.35 %). Neem leaf extract at 10 per cent (63.21 %) was found to be next best, followed by garlic bulb extract at 5 per cent (57.41 %). Neem leaf extract at 5 per cent (51.10 %) and tulsi leaf extract at 5 per cent (49.26 %) were on par with each other. Tridax leaf extract at 5 per cent (14.22 %) and 10 per cent (19.39 %) concentrations was found to be inferior to all other treatments.

B. *In vitro* evaluation of bioagents against *C. musae*:

Per cent inhibition of mycelial growth of *C. musae* by antagontstic microorganisms is presented in Table 2. The results of the experiment revealed that, highest inhibition (84.45 %) was observed in *Bacillus subtilis*, which was significantly superior to all other bioagents. *Pseudomonas fluorescens* (79.30 %) was found to be the next best bioagent, followed by *T. viride* (77.02 %), *T. virens* (isolate 2) (71.10 %), *T. harzianum* (70.30 %, *T. reesei* (69.29 %), *T. pseudokoningi* (68.89 %),*T. virens* (isolate 1) (66.05 %).

In vitro evaluation of plant extracts against *Colletotrichum musae* revealed that, neem leaf extract, Garlic bulb extract, tulsi leaf extract were effective in inhibiting the

mycelial growth of *C. musae.* The same botanicals were also effective in inhibiting the spore germination. Antifungal activity of garlic, neem and tulasi was reported earlier also. been well documented. The results obtained were in conformity with that of Ahmed and sultana (1984) who reported the antifungal activity of garlic bulb extract against *Colletotrichum corchori*. Medha Chavan (1996) also reported the antifungal activity of neem, tulsi, and garlic against the mycelial growth of *Colletotrichum gloeosporioides*. It has been demonstrated by Thoppil *et al.* (2000) that, oil extracted from tulsi has got antifungal properties against *C. musae*.

Among the biocontrol agents tested *Bacillus subtilis, Pseudomonas fluorescens, Trichoderma viride, T. virens* isolate 2, *T. harzianum* were found to be effective against *Colletotrichum musae*. Various earlier workers also have reported the effectiveness of *B. subtilis* and *P. fluorescens* against *Colletorichum spp.* Arras (1993) reported the antagonistic nature of *B. subtilis* on *C. gloeosporioides*. Chidanandaswamy (2001) reported that, *Pseudomonas fluorescens* was effective in inhibiting *C. capsici*. He also reported that, *T. viride, T. harzianum*, were also effective against *C capsici*.

From the investigations it was observed that garlic, neem and tulsi extracts were found to be effective against *C. musae*. The antifungal activity of the tulsi is reported to be due to thymol and phenol present it, which are toxic to many pathogens (Anon., 1975). Patil (1992) also reported that, extract of tulsi contains polyamine biosynthesis inhibitors(s), which block the ornithine polyamine decarboxylase pathway in *Botryodiplodia theobromae* and *Rhizopus arrhizus*. Similar mode of action may be present in other organisms too. Sharma and Prasad (1980) reported that, allicin (diallyl disulphide), allisatin I, allisatin II, garlicin, garlic phytoncide were the active principles of *A. sativum*. Antifungal activity of the garlic may be attributed to any of these compounds. Antifungal activity of neem has been reviewed in detail by, Parveen and Alam (1993). The antifungal activity of *Bacillus subtilis, Pseudomonas fluorescens, Trichoderma spp.* and other antagonistic agents can be attributed to the production of antibiotics or competition for substrate or hyperparasitism. One of these mechanisms may play an important role in suppression of *C. musae*.

Table 1: *In vitro* evaluation of botanicals against *Colletotrichum musae* of banana.

S. No	Plant extract	Percent inhibition of					
		Mycelial growth			Spore germination		
		5%	10%	Mean	5%	10%	Mean
1	Chromolaena leaf extract	25.61 (30.39)	31.15 (33.88)	28.38 (32.13)	36.71 (37.30)	45.44 (42.39)	41.08 (39.84)
2	Clerodendron leaf extract	5.82 (13.99)	13.74 (21.74)	9.78 (17.86)	21.26 (27.42)	22.57 (28.34)	21.92 (27.88)
3	Garlic bulb extract	52.21 (46.28)	68.87 (55.48)	60.03 (50.83)	57.41 (49.23)	74.00 (59.12)	65.71 (54.17)
4	Lantana leaf extract	22.62 (28.36)	32.70 (34.99)	27.66 (31.67)	36.71 (37.30)	48.54 (44.10)	42.63 (40.70)
5	Neem leaf extract	71.12 (57.48)	84.80 (67.08)	77.96 (62.28)	51.10 (45.50)	63.21 (52.85)	57.16 (49.17)
6	Parthenium leaf extract	31.10 (33.87)	33.40 (35.25)	32.25 (34.55)	26.66 (31.01)	43.01 (40.96)	34.84 (35.98)
7	Tridax leaf extract	3.56 (10.84)	7.82 (16.19)	5.67 (13.52)	14.22 (22.10)	19.39 (26.04)	16.81 (24.07)
8	Tulsi leaf extract	48.50 (44.16)	65.50 (54.07)	57.00 (49.11)	49.26 (44.59)	73.35 (58.98)	61.31 (51.78)
	Mean	29.90 (33.16)	41.00 (39.83)	35.39 (36.49)	35.90 (36.80)	48.00 (44.09)	42.10 (40.45)

Source	Sem ±	CD at 1 % Level	Sem ±	CD at 1 % Level
Plant extract (P)	0.59	2.31	0.48	1.86
Concentration (C)	0.30	1.16	0.24	0.93
PxC	0.84	3.28	0.67	2.64

*Figures in the parentheses are angular transformed values

Table 2: *In vitro* evaluation of biocontrol agents against *Colletotrichum musae* of banana.

Sl. No.	Biocontrol agent	Per cent inhibition of mycelia growth
1	*Bacillus subtilis*	84.45 (66.75)
2	*Pseudomonas fluorescens*	79.30 (62.93)
3	*Trichoderma harzianum*	70.30 (56.98)
4	*T. pseudokoningi*	68.89 (56.07)
5	*T. reesei*	69.29 (56.32)
6	*T. virens*(isolate 1)	66.05 (54.31)
7	*T. virens*(isolate 2)	71.10 (57.44)
8	*T. viride*	77.02 (61.14)
	SEm ±	0.39
	CD at 1% level	1.64

*Figures in the parentheses are angular transformed values.

Plate 1: Symptoms and spores of anthracnose of banana caused by *Colletotrichum musae*

REFERENCES

Anonymous, (1975). *The Wealth of India - Raw Maerials, Vol. IA.* Publication and Information Directorate, CSIR, New Delhi, p.513.

Ahmed, N. and Sultana, K., (1984). Fungitoxic effect of garlic on treatment of jute seed. *Bangladesh Journal of Botany.*13: 130-136.

Arras, G. (1993). Inhibition of post harvest fungal pathogens by *Bacillus subtilis* strains isolated from citrus fruit. *Advances in Horticultural Sciences*. 7:123-127.

Madan, M.S. and Ullasa, B.A., (1993). Postharvest losses in fruits. *In Advances in Horticulture*. Ed. Chadha K.L. Malhotra Publishing House, New Delhi, 4: 1795-1810.

Medha Chavan, (1996). Effect of plant extracts on some important pathogenic fungi of Konkan region. *M Sc. (Ag) Thesis*. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra.

Parveen, G. and Alam, M.M., (1993). Bioactivity against plant pathogens. In *Neem Research and Development*, Ed. Randhawa, H.S. and Parmar, B.S., Publication No.3, Society of Pesticides Science, India, pp. 144-153.

Patil, R. K., (1992). Studies on Rhizopus rot and Botryodiplodia rot of mango fruits. *Ph.D. Thesis*, Rajasthan

Agricultural University. Bikaner, Rajasthan.

Salunke, D.K. and Desai, B.B., (1984). *Postharvest Biotechnology of Fruits, Vol. I* , Boca raton, FL: CRC: p.168.

Sharma, V.D. and Prasad, D., (1980). Garlic an effective antimicrobial bulb. *Science Reporter,* 17:790.

Thoppil, J.E., Manija, J., Jajo, A. and Deena M.J.(2000). Microbicidal potential of the essential oil of *Ocimum adscendens*. *Journal Of Medicinal and Aromatic Plant Sciences*. 22:256-257.

Vincent, J.M., (1927). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature.* 159:850.