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Non-polluting way to management of fusarium fruit rot disease of banana by plant products

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Abstract
Among of different plant extract garlic cloves extract at 10 per cent was recorded highest mycelial growth inhibition (85.71%) followed by neem leaves (79.37%) and eucalyptus leaves (73.54%) extracts at same concentration under *in vitro*. Whereas, *in vivo* condition, lowest fusarium fruit rot severity (11.81%) was recorded in neem leaves extract at 10 per cent concentration and it was found at par with garlic cloves extract and tulsi leaves extract on 8th day after pre- inoculation. While in post- inoculation, lowest severity (12.18%) was recorded under the garlic cloves extract at 10 per cent concentration and it was remained at par with neem leaf extract at 10 per cent on 8th day after inoculation.

Keywords: banana, plant extarct, *Fusarium musae*, *in vitro*, *in vivo*, post-harvest

Introduction

Banana (*Musa paradisiaca* L.) is one of the most important commercial fruit crop grown all over the world in the tropical and subtropical areas. It is the second largest fruit crop, belongs to family *Musaceae* in order *Zingiberales.* It is indigenous to indo-Malayan region.

In India, banana is fourth important food crop in term of gross value exceeding only by paddy, wheat and milk products. It is also a desert fruit for million apart from a staple food owing to it's rich and easy digestibility. The ripe fruits are edible, delicious and very nutritious. The content of carbohydrates 22.84g is very high with a calorific value of 89kcal/100g fruit. It is good source of vitamin A (64 IU/100g of edible portion) and vitamin C (8.7mg/100g of edible portion) and fair source of vitamin B_1, B_2, B_3, B_5, B_6 and B_9. The fruits are rich in magnesium, sodium, potassium and phosphorus. The food value is about three times that of wheat. (Anon., 2019) [1].

Cultivated banana is susceptible to many diseases, mostly fungal pathogen which attacks various part of the plant from root to fruit. Bananas are highly perishable commodities with post-harvest losses estimated to the tune of 25-30 per cent (Kachwaha *et al*., 1991) [5]. Banana fruit suffers from many serious diseases such as fruit rot, crown rot, finger rot, cigar-end rot and pitting disease. The current postharvest problems for bananas are mainly concerned with storage and marketing. Crown rot due to, *Fusarium pallidoroseum* (Cooke) Sacc, *Colletotrichum musae* (Berk. and Curt.), *Verticillium theobromae* (Turc.) Mason and Hughes, *Thielaviopsis paradoxa* (Deseynes) Sacc, *Lasiodiplodia theobromae* (Pat.) Griffth and Maubl, *Fusarium musae* (Syd.) M. B. Ellis and *Fusarium roseum* (Link) Snyder and Hansen pathogens causes losses of bananas in grown for local consumption and export of bananas (Stark *et al*., 2008) [10]. Banana fruit majorly infected by *Alternaria alternata* (Fr.) Keissier, *Colletotrichum musae*, *Fusarium moniliforme* (Cooke) Sacc and *F. oxysporum* (Schlecht. Emend. Snyder and Hansen) pathogens. *Aspergillus flavus* Link., *A. fumigatus* Fresenius., *A. niger* Van.Teigh., *A. terreus* Thom., *Penicillium* spp. Link were dominant pathogens, same as *Curvularia lunata* (Wakker) Boedijn, *Cochliobolus lunatus* (Nelson and Haasis) and *Colletotrichum musae*. *Nigrospora oryzae* (Berk and Ba.) Petch. and *Khuskia oryzae* Hudson were active during the winter while, *Botryodiplodia theobromae* were sporadic whereas, *Deightoniella torulosa* and *Cunninghamella echinulata* (Thaxter) were detected occasionally. *Rhizoctonia solani* (Kuhn) and *Macrophomina phaseolina* (Tassi) Goid. also caused considerable damage to fruits (Sarkar *et al*., 2009).

The banana fruit infecting fungus *Fusarium musae* was originally known as a distinct population within *Fusarium verticillioides*. However, (Van Hove *et al*., 2011) [12] recently, showed, by multilocus phylogeny and mating experiments that this population represents a unique lineage in the *Fusarium fujikuroi* species complex (FFSC) and consequently they installed the new species *Fusarium musae* being closely related to (i.e. sister species) but distinct from *Fusarium verticillioides*. The first cases of human infection associated with *Fusarium musae* caused through contact with *Fusarium musae* contaminated banana fruits, either being imported or after traveling of the patient to a banana producing country. An alternative hypothesis is that *Fusarium musae* is not only present on banana fruits, but also on other plant hosts or environmental sources. In a more recent survey performed laboratory testing the feasibility of an in house developed MALDITOF MS identification assay and there by using 390 fungal isolates collected between July 2012 and July 2013 from 2 hospitals located in Brussels, one *Fusarium musae* strain was found among the 20 Fusarium isolates identified. This *Fusarium musae* strain was isolated from a blood sample of an immune-suppressed patient, whereas majority of the other fusarioses (Triest *et al*., 2016) [11].

Use of fungicide on harvested fruits to manage the diseases is not desirable from health point of view, also continuous and indiscriminate use has led to the development of fungicide resistant strains of the pathogens. It also reduces the export quality due to high residues. An attempt was made to explore the

possibility of using various phytoe-xtracts for the management of Fusarium fruit rot of banana

Materials and Methods

In vitro evaluation of phytoe-xtracts against Fusarium musae

Efficacy of different phyto-extracts of plant species having medicinal value were tested *in vitro* by Poisoned Food Technique against *Fusarium musae* (Nene and Thapliyal, 1979) [6]. The list of plant species used for phyto-extracts study is given below list indicating their scientific name, common name and plant part used. All the phyto-extracts were tested at 10 per cent concentration.

A healthy fresh part of plant *i.e.,* leaves and rhizomes were washed thoroughly with fresh water and finally rinsed with sterilized distilled water. Fifty gram of plant parts were cut into small pieces and minced with the help of a grinder by adding 50ml sterilized distilled water. The phyto-extracts were filtered through double layered muslin cloth in 150ml conical flasks and plugged with non-absorbent cotton. These filtered extracts were autoclaved at 15*lbs* pressure for 20minutes. Autoclaved extract was individually added into previously sterilized PDA plates at 10 per cent and mixed thoroughly at the time of pouring in the previously sterilized Petri plates. The Petri plates were inoculated aseptically after solidification by placing 5mm diameter mycelial disc at the center, cut aseptically with cork borer from 10 days old pure culture of causal organism. Three replications of each treatment were maintained. The plate without phyto-extracts served as control. The Petri plates were incubated at 27±2°C temperatures till the complete coverage in control plate. Each phyto-extracts at 10% were mixed thoroughly in sterilized 100ml PDA medium filled in 250ml flask under aseptic condition. The medium was supplemented with streptomycin sulphate at 50ppm to prevent bacterial contamination.

Table 1: list of plants and plant products testing under *in vitro* condition against *Fusarium musae*

Sr.No.	Common Name	Scientific Name	Parts used	Concentration (%)	Antifungal Properties
1	Garlic	*Allium sativum* L.	Cloves	10	Allicin
2	Tulsi	*Ocimum sanctum* L.	Leaves	10	Eugenol, Terpenoids, Triterpenoids
3	Babul	*Vachellia nilotica* L.	Leaves	10	Flavonoids, Sterols, Triterpenoid, Alkaloids
4	Turmeric	*Curcuma longa* L.	Rhizome	10	Cucurmin, Eugenol, Phytosterol
5	Periwinkle	*Catharanthus roseus* L.	Leaves	10	Terpenoids
6	Ardusi	*Adhatoda vasica* L.	Leaves	10	Vasicine
7	Neem	*Azadirachta indica* L.	Leaves	10	Azadirichtin
8	Acalypha	*Acalypha indica L.*	Leaves	10	Tannins, Steroids, Saponins, Lavonoids,
9	Eucalyptus	*Eucalyptus citriodora* L.	Leaves	10	Citronellol

The observations on mycelial growth (mm) and per cent growth inhibition of *Fusarium musae* recorded after 8 days of incubation. The per cent growth inhibition (PGI) of pathogen in each treatment was calculated by following formula (Vincent, 1947).

$$I = \frac{C - T}{C} X\ 100$$

Where,
I = Inhibition per cent
C = Colony diameter (mm) in control plate
T = Colony diameter (mm) in treated plate

Experimental details:

I Design : Completely Randomized Design (CRD)
II Treatments: 10 (Ten) (including control)
III Repetitions: 3 (Three)
IV Method : Poisoned food technique

In vivo* evaluation of phyto-extracts against *Fusarium musae

The Effective phyto-extracts studied *in vitro* were further tested at 10 per cent concentration of phyto-extracts by following both pre- and post-inoculation methods.

Experimental details:

I Design : Completely Randomized Design (CRD)
II Treatments: 5 (Five) (including control)
III Repetitions: 3 (Three)

Table 2: List of plants and plant products testing under *in vivo* condition

Sr. No.	Common Name	Scientific Name	Parts used	Concentration (%)
1	Garlic	*Allium sativum* L.	Cloves	10
2	Neem	*Azadirachta indica* L.	Leaves	10
3	Eucalyptus	*Eucalyptus citriodora* L.	Leaves	10
4	Tulsi	*Ocimum sanctum* L.	Leaves	10

Pre- inoculation of phyto-extracts on banana fruit

The healthy, semi-matured uniform size of banana fruits were surface sterilized by dipping in 0.1 per cent NaOCl solution for one min. followed by three washings with distilled sterile water and inoculated separately with the pathogen by the stylar-end pricking method. The fruits were first dipped in the phyto-extracts (10%) solution for15 min., air dried and then inoculated with fruit rot pathogen (10^6 spores/ml) then bagged in sterilized polythene bags with sterilized moist absorbent cotton swab. The mouth of the bag was loosely tied and incubated at 27±2°C.The untreated and inoculated and uninoculated fruits were kept as control. The interval between phyto-extracts treatment and inoculation was kept twelve hours. The severity of fruit rots was recorded on 4^{th} and 8^{th} day after inoculation with the help of assessment key.

Post- inoculation of phyto-extracts on banana fruit

The procedure mentioned in above was followed except that the fruits were first inoculated with test pathogen and then treated with phyto-extracts (10%) solution.

Assessment key used for severity of fusarium fruit rot disease of banana

Scale	Per cent infection
0	0%
1	1-10%
2	11-20%
3	21-40%
4	41-50%
5	> 60%

$$\text{Severity (\%)} = \frac{\text{Area of infected fruits}}{\text{Total area of fruit tissue}} \text{ X } 100$$

Results and Discussion

Evaluation of phyto-extracts against *Fusarium musae in vitro*

Many plant extracts are known to have an inhibitory effect on the growth and reproduction of various fungi. This information is certainly useful in exploiting the inhibitory principle in plant disease management. In the present investigation, nine phyto-extracts *viz.*, garlic cloves extract (*Allium sativum* L.), tulsi leaves extract (*Ocimum sanctum* L.), babul leaves extract (*Vachellia nilotica* L.), turmeric rhizome extract (*Curcuma longa* L.), Periwinkle leaves extract (*Catharanthus roseus* L.), ardusi leaves extract (*Adhatoda vasica* L.), neem leaves extract (*Azadirachta indica* L.), acalypha leaves extract (*Acalypha indica* L.) and eucalyptus leaves extract (*Eucalyptus citriodora* L.) at 10.0 per cent were evaluated against *F. musae* using by poisoned food technique on PDA medium. The mycelial growth and per cent inhibition of the growth of tested *F. musae* were recorded at 8th days after incubation. The results presented in table 1. All those phytoextracts were tested significantly inhibited the mycelial growth of *F. musae*. The result indicated that, mycelial growth of *F. musae* ranged from 9.00 to 35.33mm and per cent inhibition of mycelial growth of *F. musae* from 43.92 to 85.71 per cent.

Table 3: Bio-efficacy of phyto-extracts against *Fusarium musae in vitro*

Sr. No.	Natural product of plant at 10%	Mycelial Growth (mm)	Per cent Growth Inhibition (PGI)
1	Garlic cloves extract	3.08** (09.00)*	85.71
2	Tulsi leaves extract	4.53 (20.00)	68.25
3	Babul leaves extract	5.87 (34.00)	46.03
4	Turmeric rhizome extract	5.52 (30.00)	52.38
5	Periwinkle leaves extract	5.24 (27.00)	57.14
6	Ardusi leaves extract	4.64 (21.00)	66.67
7	Neem leaves extract	3.67 (13.00)	79.37
8	Acalypha leaves extract	5.99 (35.33)	43.92
9	Eucalyptus leaves extract	4.14 (16.67)	73.54
10	Control	7.97 (63.00)	----
	SEm ±	0.07	
	CD at 5 %	0.20	
	CV %	2.35	

*Figure in parenthesis is original value, **outside is square root transform value & DAI: Day after incubation

Effect of phytoextracts on colony diameter of *F. musae*

Total nine phyto-extracts at 10.0 per cent concentrations evaluated against *F. musae in vitro.* Among them, lowest mycelial growth of *F. musae* was found in garlic cloves extract with 9.00mm. The next best phytoextracts in order of merit was neem leaves extract with 13.00mm followed by eucalyptus leaves extract, tulsi leaves extract, ardusi leaf extract and Periwinkle leaves extract *i.e.*, 16.67, 20.00, 21.00 and 27.00mm mycelial growth of *F. musae*, respectively. Whereas, turmeric rhizome extract, babul leaves extract and acalypha leaves extract were found to be comparatively less effective and recorded mycelial growth of *F. musae i.e.*, 30.00, 34.00 and 35.33mm, respectively.

Effect of phyto-extracts on growth inhibition of *F. musae*

Out of nine phytoextracts, highest per cent inhibition of mycelial growth of *F. musae* was found in garlic cloves extract with 85.71 per cent. The next best phytoextract in order of merit was neem leaves extract with 79.37 per cent followed by eucalyptus leaves extract, tulsi leaves extract, ardusi leaf extract and barmasi leaves extract *i.e.*, 73.54, 68.25, 66.67 and 57.14 per cent inhibition mycelial growth of *F. musae*, respectively. Whereas, turmeric rhizome extract, babul leaves extract and acalypha leaves extract were found to be comparatively less effective and recorded minimum inhibition mycelial growth of *F. musae i.e.*, 52.38, 46.03 and 43.92 per cent, respectively.

Thus, it shows that garlic cloves extract at 10.0 per cent was found to be the more superior because it was observed lowest mycelial growth and highest per cent inhibition mycelial growth of *F. musae* as compared to rest of treatments.

From this experiment, it is very clearly shown that garlic cloves extract (*Allium sativum* L.), leaf extracts of neem (*Azadirachta indica* L.) and eucalyptus (*Eucalyptus globulus* L.) have Allicin, *azadirachtin, limonoid triterpenoid, tannin, tetranortriterpenoid* and *citronella* fungi toxic compound present in their extract which directly affects the growth of *F. musae.* Present study corroborates with many workers Singh *et al.* (1993) evaluated eleven leaf extracts of medicinal plants, the extract from *Azadirachta indica* and *Ocimum sanctum* were the most effective in inhibiting the mycelial growth of *B. theobromae*, *Fusarium oxysporum*, *Helminthosporium speciferum*, *Curvularia lunata*, *Aspergillus flavus* and *Trichothecium roseum.* The antifungal activities of cinnamon, piper and garlic extract were evaluated on banana crown rot fungi (*Colletotrichum musae*, *Fusarium* spp. and *Lasidiplodia theobromae*) *in vitro*. Cinnamon extract @ 0.5 g/l completely inhibited conidial germination and mycelial growth of all fungi (Win *et al.*, 2007). Sarkar *et al.* (2013) reported that extracts of medicinal plants *viz.*, *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum sanctum* at 30 and 50 per cent were effective in inhibiting the growth of *Nigrospora oryzae*. While, the extracts of *Allium cepa* and *Allium sativum* effectively

inhibited the pathogenic activity of *Macrophomina phaseolina*, *Fusarium oxysporum* and *Nigrospora oryzae*. Ekwere *et al.* (2015) reported that *Vernonia amygdalina* and *Azadirachta indica* at 30 per cent concentration effectively inhibited the growth of banana fruit rot causing organism when it was cultured by 76.75 and 59.44 per cent, respectively for *Aspergillus niger* and 69.34 and 58.75 per cent for *Fusarium moniliforme* after 5 days of incubation.

Evaluation of phyto-extracts against *Fusarium musae in vivo*

The effective phyto-extracts *viz.*, garlic cloves extract, neem leaves extract, eucalyptus leaves extract and tulsi leaves extract were selected based on *in vitro* performance. All four phyto-extracts were found significantly superior in reducing the fusarium fruit rot severity as compared to control on 4th and 8th day after inoculation in pre- and post-inoculation treatments (Table 2).

Pre-inoculation of phyto-extracts on banana fruit

The results revealed that significantly lowest per cent fusarium fruit rot severity was recorded in neem leaves extract 8.40 and 11.81 per cent and it was at par with garlic cloves extract 9.88 and 12.97 and tulsi leaves extract 10.70 and14.01 per cent on 4th and 8th day after inoculation. Eucalyptus leaves extract 11.93 and 18.08 per cent proved least effective in managing the fusarium fruit rot.

Post-inoculation of phyto-extracts on banana fruit

The results presented in (Table 2). Significantly lowest fusarium fruit rot severity was recorded in garlic cloves extract 7.93 and 12.18 per cent and it was at par with neem leaf extract 8.62 and 13.16 per cent on 4th and 8th day after inoculation. While tulsi leaves extract 9.91 and 15.14 per cent showed mediocre effect in reducing the fusarium fruit rot of banana. Eucalyptus leaves extract 10.87 and 15.20 per cent proved least effective in managing the fusarium fruit rot.

Thus, the results of present study corroborate with the results reported by Damaram (2013) reported that complete mycelial growth inhibition was recorded in garlic and cinnamon extract. Further, garlic clove extract (10%) was found most effective in reducing the fusarium fruit rot of tomato severity in pre-inoculation (14.17%) and cinnamon leaf extract in post-inoculation (13.66%) treatments. Jahan *et al*. (2019) reported that post-harvest application of garlic extracts at 25 per cent was recorded lower disease incidence (49.66%) and severity (11.18%) of *Colletotrichum musae* and *Lasidiplodia theobromae*, respectively thus improves the quality of stored banana fruits.

Table 4: Bio-efficacy of phyto-extracts on the severity of fusarium fruit rot of banana *in vivo*

Sr. No.	Phytoextracts (10%)	Severity (%)			
		Pre-inoculation		Post-inoculation	
		4th	8th	4th	8th
1	Garlic cloves extract	18.29** (09.88)*	21.08 (12.97)	16.33 (07.93)	20.41 (12.18)
2	Neem leaves extract	16.84 (08.40)	20.09 (11.81)	17.07 (08.62)	21.26 (13.16)
3	Eucalyptus leaves extract	20.19 (11.93)	25.16 (18.08)	19.24 (10.87)	22.93 (15.20)
4	Tulsi leaves extract	19.09 (10.70)	21.97 (14.01)	18.33 (09.91)	22.89 (15.14)
5	Untreated	23.40 (15.88)	31.27 (26.96)	22.99 (15.26)	30.27 (25.44)
	SEm ±	0.85	0.69	0.50	0.64
	CD at 5 %	2.68	2.18	1.59	2.02
	CV %	7.54	5.01	4.64	4.71

*Figure in parenthesis is original value, **outside is arcsine transform value & DAI: Day after incubation

Conclusions

Total nine phyto-extracts at 10 per cent were evaluated and found significantly superior in inhibiting the mycelial growth of *F. musae*. The highest per cent inhibition of mycelial growth was found in garlic cloves extract with 85.71 per cent. The next best phyto-extract in order of merit was neem leaves extract with 79.37 per cent followed by eucalyptus leaves extract, tulsi leaves extract, ardusi leaf extract and barmasi leaves extract *i.e.*, 73.54, 68.25, 66.67 and 57.14 per cent growth inhibition under *in vitro*, respectively. Whereas, lowest per cent fusarium fruit rot severity was recorded in neem leaves extract 11.81 per cent and it was at par with garlic cloves extract 12.97 and tulsi leaves extract 14.01 per cent on 8th day after inoculation in pre inoculation treatment. While in post inoculation lowest severity was recorded in garlic cloves extract 12.18 per cent and it was at par with neem leaf extract 13.16 on 8th day after inoculation under *in vivo*, respectively.

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