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Research Article

Antifungal activities of different plant extracts against pink mold of banana caused by *Trichothecium roseum* (Pers.) Link

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ABSTRACT: *Trichothecium roseum* (Pers.) Link causes pink mold rot in banana fruits across the world. Due to this infection, 30% of losses arise in poor man's food. To control this disease, plant extracts show an alternative to fungicides. In this study, 40 plant species were screened for their antifungal activity against *T. roseum.* Among 40 hot and cold aqueous extracts of plant species, only 10% *Allium sativum* and 6% and 8% of *Mansoa allicea* showed 100% inhibition of *T. roseum*. Freezing condition (4°C) shows 100% inhibition compared to room temperature (27°C) and incubation temperature (46°C). One hundred per cent inhibition of *T. roseum* was observed within 5 days compared to 15, 20 and 30 days of incubation. The pink mold of banana caused by *T. roseum* was reported for the first time in Karnataka.

KEYWORDS: Banana, biocontrol, pink mold, *Trichothecium roseum*, Karnataka, India

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INTRODUCTION

Banana is an elongated edible fruit - botanically a berry - produced by several large herbaceous plants in the genus Musa. In some countries, cooking bananas may be called "flour bananas" to distinguish them from dessert bananas. Fruits vary in size, colour and firmness, but are usually elongated and convex, with a soft, starchy skin that can be green, yellow, red, purple or brown when ripe. Banana varieties grown commercially in India - Grand Naine (G9), Robusta, Dwarf Cavendish, Red Banana, Nendran (Source: APEDA Agri Exchange) Commercially grown banana varieties in Karnataka – Nanjanagud Rasbale, (Mysore district and Chamarajanagar district in Karnataka) Kamalapur Red Banana, (Kalaburagi district in Karnataka), Ney Poovan, Grand Naine Banana (Archith *et al.*, 2021) Robusta Banana, Nendran, Red Banana, Gros Michel, Dwarf Plantain, Dwarf Cavendish Banana, Manzano Banana, Cavendish Banana and Ice Cream Banana In 2020-2021, India emerged as the world's largest producer of bananas, producing 297 lakh Mt on 8.4 lakh hectares of land. Domestic consumption is estimated at 290 lakh Mt (97%), while about 5 lakh Mt is lost due to the spoilage of bananas. Most of India's banana exports go to West Asian and North African countries such as the United Arab Emirates, Bahrain, Egypt, Saudi Arabia, Qatar and Iran. State Wise Banana Production Data (2021-22) Karnataka is 3713.79 Mt (11.44%) The main diseases are Panama wilt disease (*Fusarium oxysporium sp cubense*), Leaf spot and Leaf streak (*Mycospherella musicola*), Black leaf spot (*Deghtoniella torulosa)*, Brown leaf spot (*Phyllosticta musarum*), Root-knot nematodes (*Meloidogyne incognita, M. javanica*), Banana Bunchy Top Virus (BBTV) (Singh, 2013; De Waele & Davide, 1998; Kone *et al.*, 2008). In present paper, the biocontrol of fruit rotting diseases in bananas was tested with different plant extracts.

MATERIALS AND METHODS

Study area

Moodbidri is a small town situated in Mangalore taluk of central Karnataka, Latitude and longitude coordinates are 13.068799 and 74.993599. This town has a fair held on every Saturday where homegrown products are sold. During that time I visited two fruit shops in April 2018 and found symptoms as black sunken spots of various sizes on fruit. I chose two infected Robusta varieties of banana, which were infected with mycelium and brought it to the laboratory. The collected sample is incubated for 72 hours for the growth of fungal pathogens.

Isolation of fungi

The fungal pathogen was isolated from the diseased samples of banana. The Potato Dextrose Agar media (PDA) was prepared (with the following compositions i.e. Peeled potato 200 grams, Dextrose-20 grams, Agar-20 grams, and Distilled water-1000 mL) and the medium was poured into Petri plates.

After solidification of PDA medium, the 1x1mm sterilized diseased sample of banana was inoculated and incubated for 7 days at 27ºC temperature. After completing the incubation period the fungal culture was isolated and pure culture was made

Identification of fungi

The fungal colonies growing on the culture plates were identified morphologically based on their colour, type of spore, the presence of sporothecia or appresoria, colony texture, and other growth characteristics of the fungi. The isolated fungi were stained with lacto phenol cotton blue for morphological study and identification

Preparation of plant extracts

The plant materials - leaves, seeds, fruits etc. were collected. These plant parts were ground to a fine powder and were extracted with sterile distilled water (i.e., cold water and hot water extracts) and acetone following the standard method (Harbome 1984) (Figure 1B). The extract was used to study the effect of mycelia growth on PDA. The extracts of selected local plants (Table 1) were used to determine the dose-response against disease colony growth on PDA.

Determination of antifungal activity of the plant extracts

A required number of Petri plates are taken and whipped with alcohol. The Petri plates are then wrapped using aluminium foil and kept for sterilization in an autoclave at 121°C and 15psi for 20 minutes. The PDA (Potato Dextrose Agar) media is prepared by taking 200gm of potato, 20 gm dextrose and 20 gm of agar for One litre of media using one litre of distilled water. The weighed potato is boiled in water and the potato extract is taken. To the extract weighed dextrose is added and mixed well. Later the agar is added and it is boiled to dissolve the agar. When the agar is dissolved, the contents are transferred to a conical flask and it is closed using a cotton plug. An aluminium foil is placed over the cotton plug and wrapped to avoid contamination. Later on, the media is autoclaved at 121°C and 15psi for 20 mins. After autoclaving the media as well as the Petri plates are taken into the Laminar Air Flow chamber previously sterilized using 70% alcohol and the UV light kept is on for 15 mins to remove all the contaminants. The Petri plates are labelled with inoculation date, media, sample details etc. To the labelled Petri plates the media is poured and allowed to solidify. When the media on the Petri plate is solidified the isolated fungus from the sample collected is inoculated. Then the Petri plate is closed and wrapped using paraffin wax. The inoculated Petri plates are incubated for a few days for the growth of mycelia and the formation of spores. After obtaining the pure culture several subcultures are done fortnightly and stored for further studies. Selected local plants which have antifungal and antimicrobial properties are taken and extracts of leaves, fruits and seeds are taken to check the inhibition of fungal growth on PDA. The 1:1 (weight by volume) extract of each sample is done using both cold and hot water. The plant extract is prepared by grinding the sample with cold or hot water respectively and storing it in bottles. The extract is then mixed with PDA media in a ratio of 9:1 (for 10%) and poured into Petri plates which are labelled with the name of the sample type of extract and date of inoculation. A separate Petri plate labelled as control is taken with PDA without the addition of the extract. All the Petri plates are then inoculated with the fungal disc using cork borer inoculating is done at the center of each PDA plate. Incubate the plates at room temperature. Observation is done for a week and the colony diameter is measured in both the extract added plate and control. The measurement is done for a week. The inhibition of the growth of the pathogen can be determined by comparing the diameter of colonies of control and extract-added plates.

Effect of various concentrations of plant extracts

This is a method to check the concentration of extract at which the fungal growth inhibition is high. Different concentrations such as 2%, 4%, 6%, 8% and 10% of plant extract were taken and mixed with the appropriate amount of potato dextrose agar media and the *Trichothecium* was inoculated to it. The concentration at which the growth inhibition is more is noted by measuring the colony diameter as done earlier.

Effect of temperature and storage

The plant extract is stored for a month under different temperatures such as room temperature (29ºC), incubator (47ºC), and freezer (4ºC) for 30 days. The extracts were taken and inoculated for 7 days to check the activities of the plant extracts under different temperatures and storage conditions.

RESULTS

Isolation and identification of fungi

Trichothecium roseum was isolated from the surface of incubated (37°C) banana fruits (Figure 1A). The isolated fungus was inoculated on a sterile PDA media and incubated at lab temperature. After 3-4 days the spores from the outer surface of mycelium were isolated and subjected to subcultures

Table 1. Plants used for screening for their antifungal activity against *T. roseum* on PDA Media

Sl. No.	Name of the species	Family	Part used
1	*Adathoda vesica* L.	Acanthaceae	Leaves
2	*Alium sativum* L.	Lilliaceae	Bulb
3	*Andrographis paniculata* (Burm f.) *wall*	Acanthaceae	Leaves
4	*Areca catechu* L.	Palmae	Rind
5	*Azadirachta indica* A. Juss	Meliaceae	Leaves
6	*Calotropis gigantia* L.	Asclepediaceae	Leaves
7	*Calycopteris floribunda* (Roxb.) Lam	Combretaceae	Leaves
8	*Capsicum annuum* L.	Solanaceae	Fruit
9	*Catharanthus roseus* L.	Apocyanaceae	Leaves
10	*Chromolaena odorata* (L.) R. M. King & H. Rob	Asteraceae	Leaves
11	*Cinnamomum zeylanicum* J. Presl	Lauraceae	Leaves
12	*Citrus limon* (L.) Osbeck	Rutaceae	Leaves
13	*Citrus limon.* (L.) Osbeck	Rutaceae	Fruit
14	*Colacasia esculanta* (L.) Schott	Araceae	Leaves
15	*Costus speciosus* (J. Konig) C. Spechl	Zingiberaceae	Leaves
16	*Curcuma longa* L.	Zingiberaceae	Rhizome
17	*Cymbopogon citrates.* (D. C.) Stapf	Poaceae	Leaves
18	*Cynodon dactylon* L.	Poaceae	Leaves
19	*Cyperus rotundus* L.	Cyperaceae	Leaves
20	*Eclipta prostate* L.	Compositae	Leaves
21	*Garcinia indica* Choisy	Clusiaceae	Leaves
22	*Holigarna ferrruginea* Buch. Ham	Anacardiaceae	Leaves
23	*Jasminum malabaricum* Wight	Oleaceae	Leaves
24	*Lantana camera* L.	Verbenaceae	Leaves
25	*Lawsonia inermis* L.	Lythraceae	Leaves
26	*Leea indica* (Burm f.) Merr	Vitaceae	Leaves
27	*Leucas linifolia* L.	Lamiaceae	Leaves
28	*Mangifera indica* L.	Anacardiaceae	Leaves
29	*Manilkara zapota (*L.) P. Royen	Sapotaceace	Leaves
30	*Mansoa alliacea* (Lam.) A. H. Gentry	Bignoniaceae	Leaves
31	*Momordica charantia* L.	Cucurbitaceae	Fruit
32	*Ocimum sanctum* L.	Lamiaceae	Leaves
33	*Passiflora foetida* L.	Passifloraceae	Leaves
34	*Phyllanthus amarus.* L.	Phyllanthaceae	Leaves and fruits
35	*Piper betle* L.	Piperaceae	Leaves
36	*Plectranthus ambonicus* (Lour.) Spreng	Lamiaceae	Leaves
37	*Tecoma stans* (L.) Juss	Bignoniaceae	Leaves
38	*Tinospora cordifolia* (Thunb.) Miers	Menispermaceae	Leaves
39	*Zingiber officinale* Roscre	Zingiberaceae	Rhizome
40	*Ziziphus jujube* Mill	Ramnaceae	Leaves

to get the pure culture. Slant cultures were also maintained as stocks of the fungus to be used for future studies.

Determination of antifungal activity of the plant extracts

Results of *in vitro* evaluation of plant extracts for their antifungal effect on the mycelia growth of the pathogen (Table 2).

The effect of cold and hot water extracts of plants at 10% concentration on the radial growth of *T. roseum in vitro* on PDA medium was studied and the results are presented in Table 2.

Out of 40 samples, 39 screened plants *Allium sativa* and *Manosa allicea* showed 100% inhibition of the growth

Table 2. The percentage of inhibition of *T. roseum* by cold and hot water plants extracts

Sl. No.	Name of the plant species	Percentage of growth inhibition of *T. roseum* over respective control			
		24 hours		48 hours	
		Hot	Cold	Hot	Cold
1	*Adathoda vesica* L.	40.1	41.3	38.7	39.7
2	*Alium sativum* L.	100	100	100	100
3	*Andrographis paniculata* (Burm f.) wall	63	65.1	62.7	64.9
4	*Areca catechu* L.	37	36.9	36.5	37.3
5	*Azadirachta indica* A. Juss	53	57	52.2	54.1
6	*Calotropis gigantia* L.	3.1	3.7	2.9	2.93
7	*Calycopteris floribunda* (Roxb.) Lam	19.9	23.5	19.1	22.7
8	*Capsicum annuum* L.	91	92	89	89
9	*Catharanthus roseus* L.	57	59.9	56	57.7
10	*Chromolaena odorata* (L.) R. M. King & H. Rob	23	24.7	20.1	19.7
11	*Cinnamomum zeylanicum* J. Presl	49.5	51.1	47.3	49.3
12	*Citrus limon* (L.) Osbeck	57	56.5	55.5	56.7
13	*Citrus limon* (L.) Osbeck	83.7	84.9	80.1	81.1
14	*Colacasia esculanta* (L.) Schott	3.1	4.3	2.8	2.9
15	*Costus speciosus* (J. Konig) C. Spechl	0.7	0.9	0.6	0.7
16	*Curcuma longa* L.	43	49	42.9	48.3
17	*Cymbopogon citrates* (D. C.) Stapf	83	85	81.2	83.5
18	*Cynodon dactylon* L.	13.7	14.1	12.9	11.8
19	*Cyperus rotundus* L.	10.1	9.7	9.3	7.9
20	*Eclipta prostate* L.	57	58.7	56.5	57.3
21	*Garcinia indica* Choisy	37	39.3	36.7	38.7
22	*Holigarna ferruginea* Buch. Ham	47	48.7	45.5	47.3
23	*Jasminum malabaricum* Wight	27	27	26	25.5
24	*Lantana camera* L.	33	35.1	31	32.7
25	*Lawsonia inermis* L.	83	89.3	82.1	87.4
26	*Leea indica* (Burm f.) Merr	77	79	76.1	78.3
27	*Leucas linifolia* L.	47	49.1	45.7	48.3
28	*Mangifera indica* L.	20.1	21.7	20.1	21
29	*Manilkara zapota* (L.) P. Royen	23.5	26.1	22.7	25.3
30	*Mansoa alliacea* (Lam.) A. H. Gentry	100	100	99.7	100
31	*Momordica charantia* L.	18.3	19.9	15.7	15
32	*Ocimum sanctum* L.	36.3	39.1	33.1	35.2
33	*Passiflora foetida* L.	10.1	12.3	9.8	11.4
34	*Phyllanthus amarus.* L.	27	26..3	25.1	27.3
35	*Piper betle* L	13.7	14.1	12.3	13.4
36	*Plectranthus ambonicus* (Lour.) Spreng	33.7	39	30.3	35.1
37	*Tecoma stans* (L.) Juss	10.3	11.4	5.6	7.3
38	*Tinospora cordifolia* (Thunb.) Miers	57	59	55	56.5
39	*Zingiber officinale* Roscre	81.7	85	80.3	81.7
40	*Ziziphus jujube* Mill	21.3	22.5	13.5	14.7

Table 3. Effect of plant concentrations on inhibition of pink mold of banana

Sl. No.	Name of the plant species	Percentage of growth inhibition of *T. roseum* over respective control				
		2%	4%	6%	8%	10%
1	*Allium sativum*	99.7	99.8	100	100	100
2	*Mansoa allicea*	100	100	100	100	100

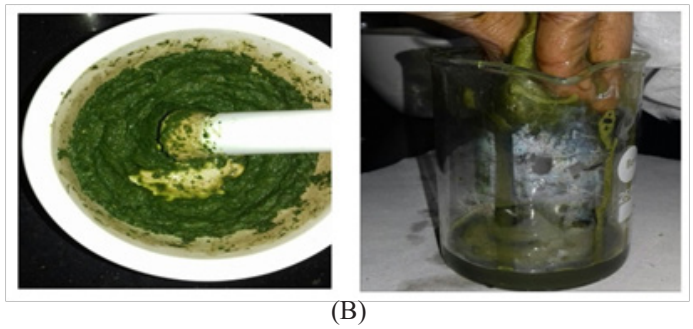
Table 4. Effect of temperature on inhibition of pink mold of banana

Sl. No.	Name of plant species	% of growth inhibition of *T. roseum* over respective control		
		Freezer (4°)	Room temperature(27°)	Incubator (46°)
1	*Allium sativum*	100	95.3	93
2	*Mansoa allicea*	100	98.1	95

Table 5. Effect of incubation period on inhibition of pink mold of banana

Sl. No.	Name of the plant species	Percentage of growth inhibition of *T. roseum* over respective control			
		5 days	15 days	20 days	30 days
1	*Allium sativum*	100	95.7	93.1	89.3
2	*Mansoa allicea*	100	100	95.7	90.8

(A)



(B)

Figure 1(A). Isolation of fungal pathogen from infected banana 40X. **1(B).** Preparation of plant extract by using mortar and pestle.

of *T. roseum.* These plants were subjected to the effect of concentration, the effect of temperature and the effect of storage.

The effect of the concentration of plant extracts at 2%, 4%, 6%, 8% and 10% concentration on the growth of *T. roseum in vitro* on PDA medium was studied and results are presented in Table 3. As the percentage concentration of plant extract increasing the inhibition of pink mold of banana also increasing. 2% concentration of both *A. sativum* and *M. allicea* shown 100% inhibition of pink mold (Figures 2 and 5).

The effect of temperature on the plant extracts was studied and their effect on radial growth of *T. roseum in vitro* on PDA medium was observed and results are tabulated in Table 4. As temperature increasing the percentage inhibition of pink mold also decreasing, so cold temperature 4°C is more suitable for control of pink mold of banana (Figures 3 and 6).

The effect of storage on the plant extracts was studied and its effect on radial growth of *T. roseum in vitro* on PDA medium was observed and results were presented in Table 5. As the incubation period increasing the percentage inhibition of pink mold of banana is decreasing, so 5 days of incubation able to show 100% inhibition of pink mold (Figures 4 and 7).

DISCUSSION

The review includes the use of cold storage as the main physical method for reducing biotic and abiotic diseases. Physical treatments like heat treatment, including hot water and hot air treatments, and radiofrequency are treated as promising control methods for postharvest diseases of fresh fruits like bananas (Usall *et al.,* 2016) Post harvests diseases of fruits and vegetables include a variety of rots and decay caused by bacteria and fungi. These organisms may cause soft rots or light brown lesions on fruits and vegetables. Being highly perishable fruit, banana suffers post-harvest losses both in terms of quality and quantity. *T. roseum* is a fungus in

Figure 2. Effect of concentration of *Mansoa allicea* plant extract on *T. roseum* for 1-7 days.

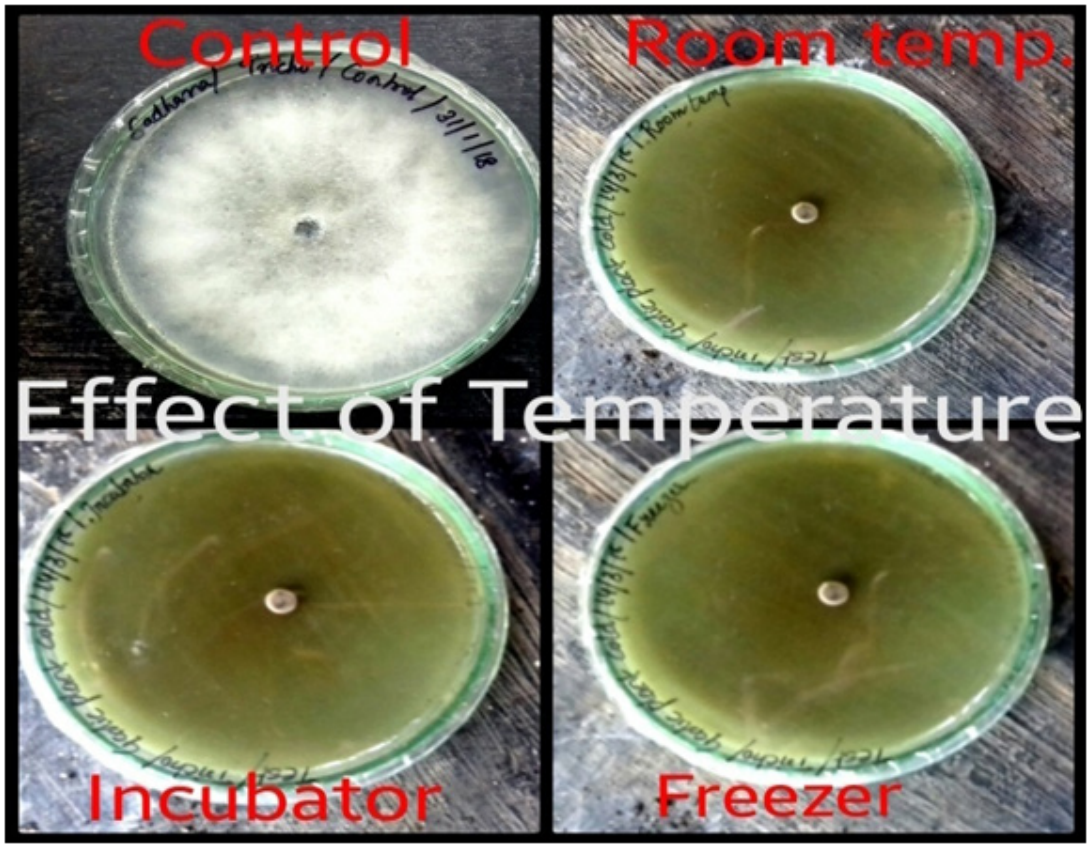


Figure 3. Effect of temperature on *M. allicea* plant extract and its effect on *T. roseum* for 1-7 days.

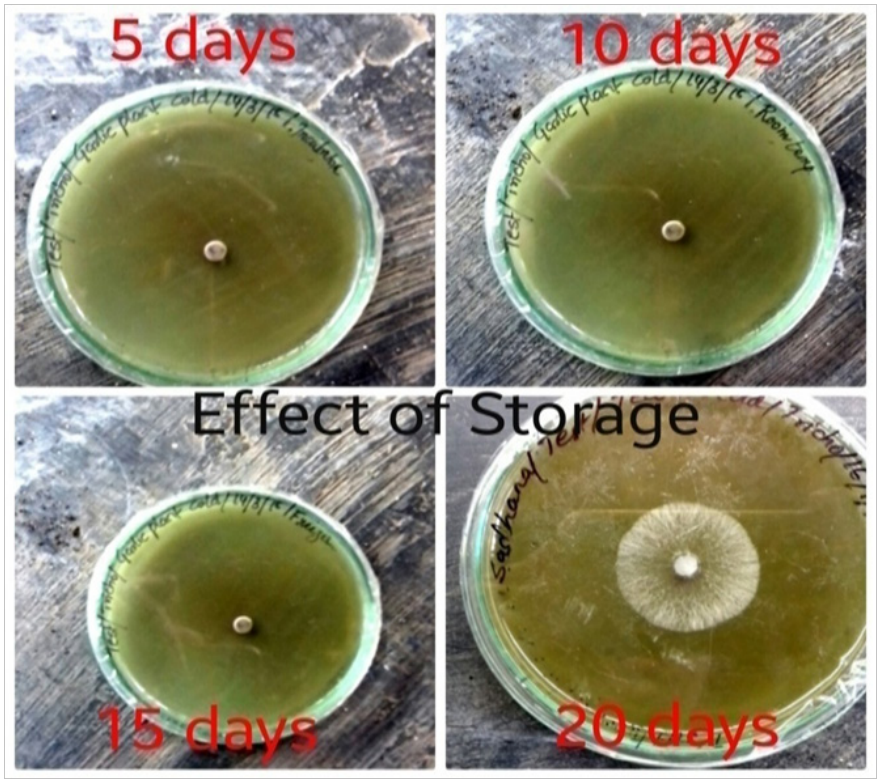


Figure 4. Effect of storage on *M. allicea* plant extract and its effect on *T. roseum* for 1-7 days.

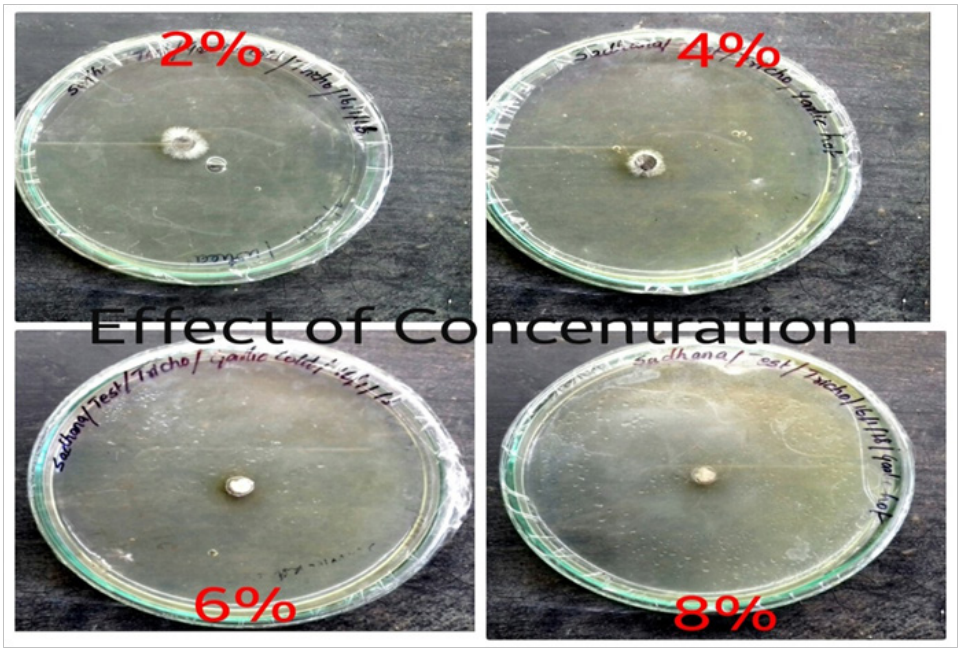


Figure 5. Effect of concentration of *A. sativum* plant extract on *T. roseum* for 1-7 days.

the division Ascomycota and is found to be one of the causal organisms of this soft rot of Banana. Physical treatments have gained great interest in recent years to control many post-harvest diseases in bananas because of the total absence of residues in the treated products and minimal environmental impact. The present review shows the extensive research work conducted over many years, developing physical means for consistent disease control. But these methods damage the fruit quality, hence chemical treatments are used. However, the use of systematic fungicides leads to residual toxicity, health hazards and atmosphere pollution. Hence many alternative methods have been attempted for the effective

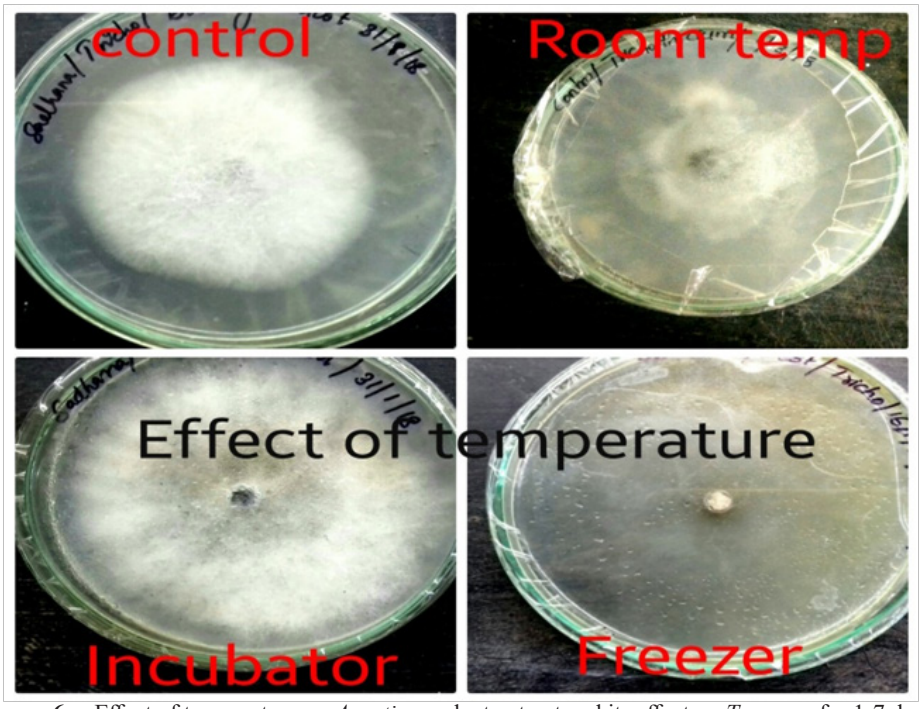


Figure 6. Effect of temperature on *A. sativum* plant extract and its effect on *T.roseum* for 1-7 days.

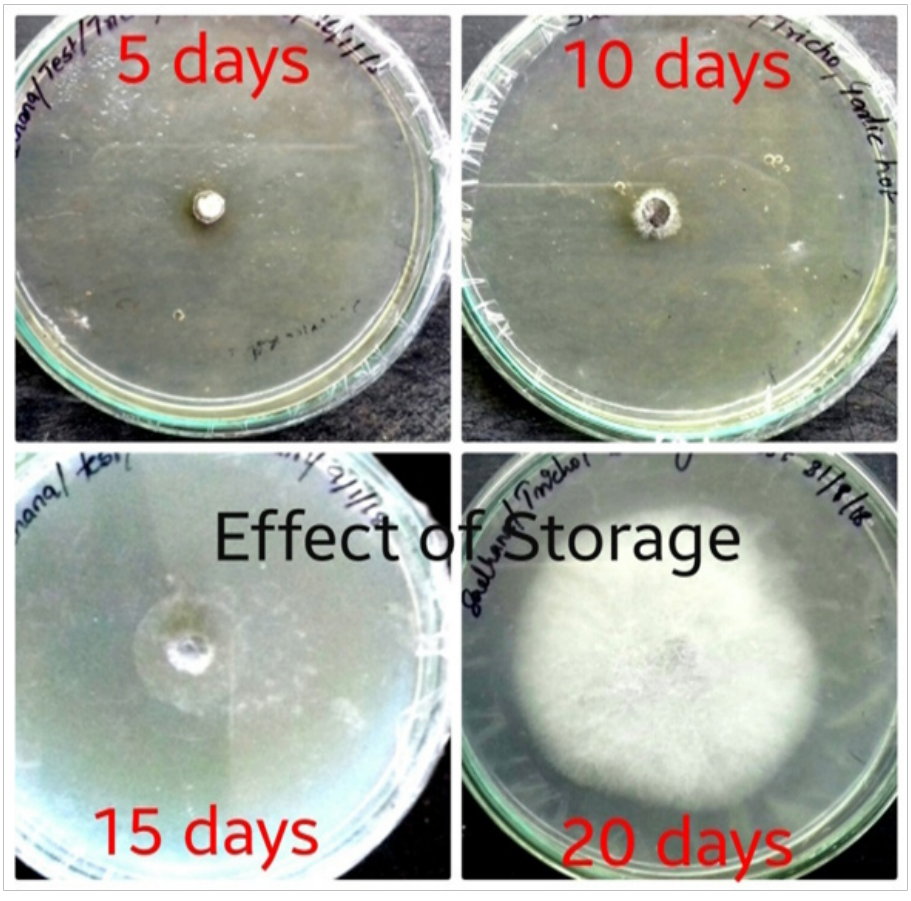


Figure 7. Effect of storage on *A. sativum* plant extract and its effect on *T. roseum* for 1-7 days.

management of the disease. Biological control of post harvest disease is one such method. Another method which is being tested currently in a large number of laboratories for the control of plant diseases is by using botanical pesticides. This has been gaining importance and recognition in recent years due to several reasons. Plant products have been reported to be easily biodegradable, specific to the targeted pathogen, harmless to beneficial organisms or antagonistic organisms and also less prone to the development of resistance by the pathogen. The plant products are also easily available locally to the farmers which can be used conveniently.

CONCLUSIONS

In present days the use of botanical pesticides has been gaining recognition and importance for various reasons. The pesticides extracted from the plants are more likely to be easily biodegradable, found to be target specific in the pathogen, they cause no harm to antagonistic organisms and even pathogen has less chance of becoming pesticide resistance because pesticides of plant origin are complex molecules. So, botanical pesticides are employed as an alternative method. Hence plant extracts had effective action on the conservation and disease control of post-harvest Banana fruit. First, losses in biomass, diameter and fruit length were lower in the *Allium sativum* extract and these treatments preserved the best peel colour. Second, lower incidence, disease severity and the highest percentage of soft rots were observed in the extract of *Mansoa allicea*. Control was observed after using these natural products. Among 39 aqueous plant species, only extracts from *A. sativum* and *M. allicea* at 10% concentration, completely inhibited the mycelia growth of *T. roseum* in PDA plates at 48 hours of inoculation. *M. allicea* is found to be more effective even at lower concentrations of 6% and 8%.

The extract of *M. allicea* was effective even at 2% concentration whereas the extract of *Allium* was found to lose its effect below 4%. The studies revealed that a temperature above room temperature is not suitable for storing the botanical pesticide because it not only loses its effect but also supports the growth of sporophytic fungi. The extract of *M. allicea* can be stored for about 20 days without reduction in the efficiency to inhibit the growth but the extracts of *Allium* can only be stored for about 10 days retaining its effect on radial growth of mycelium of *T. roseum.*

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