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Eco-Friendly Management of Penicillium Fruit Rot of Citrus

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Abstract
Citrus, the third most important fruit crop after mango and banana, suffers significant post-harvest losses due to *Penicillium* species, particularly *P. digitatum*. A survey (Jan-Apr 2021) in Dudia Talav market, Gujarat, identified *Penicillium* rot as the predominant issue, with peak incidence in mid-February. Diseased fruits were sampled, and the pathogen was identified based on cultural and microscopic traits. On potato dextrose agar, *P. digitatum* formed fast-growing, olive-green, velvety colonies with irregular margins and pale-yellow pigmentation beneath. Mycelium was hyaline, septate, and branched. Conidiophores were terverticillate, and conidia were smooth, green, ellipsoidal to cylindrical (6.0-12.5 × 3.5-6.0 µm). Among biocontrol agents tested, *Pseudomonas fluorescens* showed the highest inhibition (93.13%), followed by *Trichoderma harzianum* (86.18%) and *T. viride* (79.83%). Turmeric was the most effective plant extract, inhibiting pathogen growth at all tested concentrations (5%, 10%, 15%), followed by garlic. Edible chemicals *viz*., sodium bicarbonate, potassium metabisulphite, and calcium chloride completely inhibited mycelial growth at 750 ppm both *in vitro* and *in vivo*.

Keywords: Eco-friendly management, penicillium fruit rot, citrus

1. Introduction
Citrus, a major fruit crop of international importance from the family Rutaceae, ranks as the third most significant fruit crop in India after mango and banana. Native to Southeast Asia, its key cultivars include mandarin, sweet orange, grapefruit, pomelo, lime, and lemon. Citrus fruits are valued for their high juice content, unique taste, and nutritional value, being non-climacteric with a relatively long shelf life. Optimal growth conditions include tropical and subtropical humid regions, full sunlight, and temperatures ranging from 13-35 °C. In India, major citrus-producing states include Maharashtra, Rajasthan, Madhya Pradesh, Karnataka, Tamil Nadu, and Manipur, while Gujarat ranks eighth, contributing 385.6 million fruits annually over an area of 37.1 thousand hectares.

Citrus fruits are widely consumed fresh, as juice, or in confectioneries, and are rich in energy, vitamins, minerals, and dietary fiber (Kumar and Shrivastava, 2012). They contain high levels of antioxidants such as vitamin C (38.5 mg/100 mL), flavanones, and anthocyanins. Despite their acidic pH (2.0-2.4), which inhibits bacterial growth, citrus fruits are highly susceptible to fungal decay during post-harvest handling, transport, and storage due to their high-water content and nutrient composition (Talibi *et al*., 2014) [15]. Citrus fruits are highly susceptible to post-harvest fungal pathogens during transportation and storage, leading to significant quality and quantity losses. In developing countries with limited refrigeration and transport facilities, post-harvest diseases can cause losses of 20-40% (Anonymous, 2018) [1].

Contamination and infection by pathogenic fungi occur at different stages after harvest and usually follows mechanical injury of the fruit, which allows entry of these organisms. Over 20 kinds of diseases occurring in citrus during post-harvest period has been reported so far. Among them green and blue moulds caused by *Penicillium digitatum* Sacc., and *Penicillium italicum* Wehmer, are most devastating and they easily deteriorate fruit by rotting.

At room temperature, green mold tends to colonize fruit significantly faster than blue mold and typically dominates in cases of mixed infections. Both pathogens, which are wound invaders, generate abundant airborne spores (conidia) through asexual reproduction, gaining

entry into fruit via wounds caused by insects, branches, or improper human handling during harvesting and transportation (Kellerman *et al*., 2016) [7]. The cycle of infection and sporulation can occur multiple times throughout the season, particularly in packing facilities. While the fruit rot rate generally ranges between 10-30%, it can escalate to as much as 50% under adverse conditions, resulting in substantial economic losses. *Penicillium* species also been reported to produce toxic secondary metabolites such as patulin and citrinin which are responsible to have negative effects on human health (Yu *et al*., 2020) [19].

Control of post-harvest diseases in citrus relies heavily on synthetic fungicides like imazalil, pyrimethanil, fludioxonil, and thiabendazole. However, their use raises concerns, including human health risks, environmental contamination, reduced fungicide efficacy due to resistant fungal strains, and restricted access to organic markets demanding lower pesticide levels (Palou, 2018) [13]. Consequently, safer, eco-friendly alternatives, such as plant extracts, medicinal plant extracts, citrus extracts, and microbial antagonists, are gaining attention for disease management. This study aims to assess the incidence of *Penicillium* rot in citrus, identify the pathogen, and explore eco-friendly management methods.

2. Materials and Methods

2.1 Collection, isolation and purification of the pathogen

Citrus fruits with typical rotting symptoms were collected from Dudia Talav market, Navsari. Diseased samples were surface sterilized with 0.1% sodium hypochlorite for 1 minute, rinsed with distilled water, and plated on potato dextrose agar (PDA). After incubation at 28 ± 2°C for 7 days, fungal growth was sub-cultured using the hyphal tip method for purification. The pure culture was maintained on PDA slants for further analysis.

2.2 Pathogenicity Test

To confirm the pathogenicity of the fungus, two inoculation methods were employed. In the Pin Prick Method, healthy citrus fruits were surface sterilized with 1% sodium hypochlorite and washed with distilled water. Sterilized pins were used to make small injuries on the fruit surface, and a spore suspension (10^6 spores/mL) was applied to the wounds. In the Deep Wound Method, fruits were disinfected with 1% sodium hypochlorite, rinsed, and dried. A sterile blade created wounds around the equatorial region, and the pathogen was inoculated into these wounds.

2.3 Cultural and morphological characterization

Cultural characterization of *Penicillium digitatum* was conducted by placing a 5mm mycelial disc from a seven-day-old culture onto sterilized PDA in Petri plates, followed by incubation at 28 ± 2°C for seven days. Observations on mycelial growth, colony color, and diameter were recorded. For morphological characterization, a small amount of pure culture was transferred to a glass slide from four positions on the culture plate. Measurements of conidia length and width, and conidiophore size and shape were taken using a light microscope at 40X magnification, with ten observations per characteristic, and mean values were calculated.

2.4 Eco-friendly management of penicillum fruit rot of citrus

2.4.1 Evaluation of antagonists against *penicillium in vitro*

In vitro evaluation of biocontrol agents was performed using the dual culture technique. The *in vitro* evaluation of various antagonists against *Penicillium digitatum* involved the following treatments: *Trichoderma viride* (T_1), *Trichoderma harzianum* (T_2), *Bacillus subtilis* (T_3), and *Pseudomonas fluorescens* (T_4), with a control group (T_5). The experiment was conducted using a Completely Randomized Design (CRD) and included four replications for each treatment. A 5mm mycelial disc of the biocontrol agent and the pathogen was placed on the periphery of the Petri plate with a 60mm distance between them. For bacterial biocontrol agents, the plate was streaked, and the pathogen disc was placed at the corner. In the control, only the pathogen was placed in the centre. Plates were incubated at 27 ± 1 °C for seven days. Mycelial growth and percent growth inhibition (PGI) of the pathogen were recorded. PGI was calculated using the formula, Vincent, 1947 [17].

$$PGI=(DC-DT)/DC\times100$$

where PGI = Per cent growth inhibition, DC is the average colony diameter of the control (mm), and DT is the average colony diameter of the treated plate (mm).

2.4.2 Effect of plant extracts against fruit rot pathogen *in vitro* and *in vivo*

The efficacy of plant extracts against the fruit rot pathogen *Penicillium* was tested both *in vitro* using poisoned food technique and *in vivo*. *In vitro*, plant parts (100g) were washed, crushed with water, and filtered to prepare 1:1 extract, which were mixed with sterilized PDA medium at concentrations of 5, 10, and 15ml. Petri plates were inoculated with a 5mm mycelial disc of the pathogen, and mycelial growth and percent inhibition were recorded after seven days of incubation. *In vivo*, effective extracts were applied to disinfected, wounded citrus fruits. The wounded fruits were dipped in botanical solution and the control fruit was treated with same volume of sterile distilled water. After two hrs., of incubation at room temperature, each wound was inoculated with 25µl of spore suspension (10^6 spores/mL) of Penicillium, the fruits were stored for ten days, and disease severity was assessed. The botanical extract used are listed in Table 1 with CRD design and 3 replications.

Table 1: *In vitro* evaluation of different plant extracts against *P. digitatum*

Treatments	Name of the Plant extract	Botanical name	Plant part used	Concentrations (%)		
T_1, T_2, T_3	Turmeric	*Curcuma longa* L.	Rhizomes	5	10	15
T_4, 5, T_6	Neem	*Azadiracta indica* L.	Leaves	5	10	15
T_7, T_8, T_9	Tulsi	*Ocimum sanctum*	Leaves	5	10	15
T_{10}, T_{11}, T_{12}	Garlic	*Allium sativum* L.	Cloves	5	10	15
T_{13}, T_{14}, T_{15}	Aloe vera	*Aloe barbadens* L.	Leaves	5	10	15
T_{16}, T_{17}, T_{18}	Eucalyptus	*Eucalyptus amygdalina* L.	Leaves	5	10	15
T_{19}	Control	-		-	-	-

2.4.3 Effect of edible chemicals on penicillium growth *in vitro* and *in vivo*

The impact of edible chemicals on *Penicillium* growth was evaluated both *in vitro* and *in vivo*. For the *in vitro* assessment, edible chemicals were mixed with PDA containing streptocycline at 250, 500, and 750ppm, and 5mm mycelial discs of the pathogen were placed on the plates. After seven days of incubation at 25 ± 1 °C, the mycelial growth and percent inhibition were measured. *In vivo*, the six most effective edible chemicals from *the in vitro* tests were applied to wounded citrus fruits, and disease severity was recorded on the eighth day, following the same method as used for plant extracts. The list of different chemicals used are listed in Table 2 with CRD design and 3 replications.

Table 2: *In vitro* evaluation of different edible chemicals against *P. digitatum*

Treatments	Name of the edible chemicals	Concentrations (ppm)		
T_1, T_2, T_3	Sodium bicarbonate	250	500	750
T_4, T_5, T_6	Potassium metabisulphite	250	500	750
T_7, T_8, T_9	Sodium chloride	250	500	750
T_{10}, T_{11}, T_{12}	Calcium chloride	250	500	750
T_{13}	Control	-	-	-

3. Results and Discussion

3.1 Survey for Penicillium Fruit Rot Incidence at Markets of Navsari

The weekly survey was carried out from first week of January to fourth week of April, 2021. The survey revealed the presence of Penicillium fruit rot pathogen (*Penicillium digitatum*) at Dudia Talav, Viraval and Ganadevi markets of Navsari. The data is presented in Table 3.1. Verma (2009) [16] observed that major fruit spoilage of Kinnow mandarins during the months of November to April was dominated by incidence of *P. digitatum* (5.18- 8.85%) and *P. italicum* (2.59-4.82%) compared to remaining post-harvest fungal rot pathogens.

Table 3: Incidence (%) of Penicillium fruit rot in markets of Navsari

Sr. No.	Incidence of fruit rot in markets of Navsari				
	Month-Week	Dudia Talav	Ganadevi	Viraval	Mean
1	January- I	12	12	10	11.33
2	II	16.7	10	12	12.90
3	III	15	13.3	6.7	11.67
4	IV	13.3	8	8	9.76
5	February - I	25	23.3	16	21.43
6	II	23.3	24	20	22.43
7	III	26.7	20	16.7	21.13
8	IV	20	16.7	15	17.23
9	March - I	16.7	13.3	15	15.00
10	II	12	12	10	11.33
11	III	16	15	12	14.33
12	IV	15	16	13.3	14.76
13	April - I	12	8	10	10.00
14	II	10	6.7	8	8.23
15	III	8	5	5	6.00
16	IV	5	4	6.7	5.23

3.2 Isolation, purification and Pathogenicity test of fungal pathogen

The fungal pathogen responsible for citrus fruit rot was isolated from diseased samples collected at Dudia Talav market, Navsari, Gujarat. The tissue isolation technique was used to obtain a pure culture of *Penicillium digitatum* from naturally infected fruits. This pure culture was maintained on fresh PDA slants and stored under refrigeration for further investigation. The pathogenicity test of *P. digitatum* was carried out on healthy citrus fruits under *in vitro* conditions following both pin prick and deep wound methods. The results indicated that the symptoms appeared on the fruits after six days of inoculation which were identical to the symptoms appeared under natural infection. Pathogen re-isolated from such inoculated fruits was identical to the original pathogen thereby proving Koch's postulates.

3.3 Cultural and morphological characteristics

The cultural characteristics of *P. digitatum* were studied on PDA medium incubated at 27 °C. Colonies appeared olive green, fast-growing, irregularly shaped, and velvety to powdery in texture, with a diameter of 68mm and visible conidiophores and conidia (Photo 1). These findings align with earlier studies. El-Gali (2014) [3] observed *P. digitatum* forming radial colonies with white airy mycelia and olive-green substrate mycelium on PDA, displaying a clear zone at the plate's bottom. Similarly, Khan *et al.* (2015) [8] reported bluish-green, fast-growing colonies with a powdery texture.

The morphological characterization of *P. digitatum* revealed hyaline, septate, branched mycelium with terverticillate conidiophores arising from subsurface or aerial hyphae. Cylindrical phialides (10.5-15.0 × 3.5-5.0 µm) produced smooth, green, ellipsoidal to cylindrical conidia (6.0-12.5 × 3.5-6.0 µm) (Photo 2). These findings align with standard descriptions by Khokhar *et al.* (2013) [9], and Rasool *et al.* (2014) [14].

3.4 Eco-friendly management of *Penicillum* fruit rot of citrus

3.4.1 *In vitro* evaluation of bio agents against *Penicillium digitatum*

Biocontrol agents provide an effective, economical, and eco-friendly approach to managing diseases without harming plants, the environment, or applicators. *In vitro* evaluation of native antagonists (*Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis*) against *Penicillium digitatum* using dual culture techniques showed significant pathogen suppression. *P. fluorescens* exhibited the highest inhibition (93.13%) with minimal fungal growth (2.58 mm), followed by *T. harzianum* (86.18%), *T. viride* (79.83%), and *B. subtilis* (64.24%) shown in Photo 3, Figure 1. These findings align with earlier studies by Wang *et al.* (2018) [18], and Ferreira *et al.* (2020) [5], which highlighted the role of antagonists in inhibiting fungal growth via rapid nutrient uptake and antifungal compound production.

3.4.2 Effect of plant extracts against *Penicillium digitatum in vitro*

Six plant extracts, including turmeric, neem, tulsi, garlic, aloe vera, and eucalyptus, were tested at concentrations of 5%, 10%, and 15% to assess their *in vitro* efficacy against *Penicillium digitatum* using the standard poisoned food technique (Nene and Thapliyal, 1979) [11]. Observations on average colony diameter and percentage inhibition of linear growth are summarized in Table 3.2 and illustrated in Photo

4. At all tested concentrations (5%, 10%, and 15%), turmeric consistently showed the highest efficacy against *Penicillium digitatum*, with the lowest mycelial growth (2.59-3.06 mm) and the greatest growth inhibition (90.18-93.11%). Garlic ranked second, with growth inhibition ranging from 83.92% to 89.63%. In contrast, extracts of tulsi, neem, aloe vera, and eucalyptus demonstrated comparatively lower efficacy, with growth inhibition below 32% even at higher concentrations. A positive correlation was observed between plant extract concentration and fungal growth inhibition across all treatments. These findings highlight the superior antifungal potential of turmeric and garlic, as supported by the findings of Obagwu and Korsten (2003) [12] and Jhalegar *et al*. (2014) [6], who similarly identified the effectiveness of garlic and aloe vera extracts against *P. digitatum* and related pathogens.

Table 4*: In vitro* evaluation of plant extracts against *Penicillium digitatum*

Sr. No.	Name of the plant extracts	Concentration (%)	Average colony diameter (mm)#	Per cent growth inhibition
1	Turmeric	5	3.06* (8.83) **	90.18
		10	2.86 (7.67)	91.47
		15	2.59 (6.20)	93.11
2	Neem	5	8.70 (75.17)	16.47
		10	8.16 (66.17)	26.47
		15	7.95 (62.73)	30.30
3	Tulsi	5	8.68 (74.83)	16.85
		10	8.56 (72.83)	19.07
		15	8.15 (65.87)	26.81
4	Garlic	5	3.87 (14.47)	83.92
		10	3.51 (11.83)	86.85
		15	3.14 (9.33)	89.63
5	Aloe vera	5	8.81 (77.13)	14.3
		10	8.34 (69.00)	23.33
		15	7.88 (61.57)	31.58
6	Eucalyptus	5	9.13 (82.77)	8.03
		10	8.32 (68.73)	23.63
		15	7.94 (62.53)	30.52
7	Absolute control	-	9. 5 1 (90.00)	-
S. Em. ±		0.33		
C. D. a t 5%		0.93		
C.V.%		1.09		

#Average of three repetitions
*Figures outside parenthesis are √x+0.5 transformed value
**Figures in parenthesis are original values

3.4.3. Effect of plant extracts against the pathogen *in vivo*
Phyto-extracts demonstrating efficacy *in vitro* were further assessed *in vivo* on citrus fruits using the fruit dip method at varying concentrations. Disease severity data was recorded to calculate the percent disease index (PDI) and decay reduction index (DRI) for each treatment (Table 3.3, Photo 5). All treatments effectively controlled the pathogen, achieving complete inhibition of *Penicillium* fruit rot compared to the untreated control after inoculation at 25 ± 1°C. Similar findings were reported by Daniel *et al*. (2015) [2], who evaluated garlic extracts and clove oil for their ability to reduce decay caused by *Botrytis cinerea*, *Penicillium expansum*, and *Neofabraea alba* on apple cultivars, demonstrating reduced lesion diameters relative to controls.

Table 5: *In vivo* evaluation of plant extracts against *P. digitatum*

Sr. No.	Name of the plant extracts	Concentration (%)	Per cent disease index (PDI)#	Decay reduction index (DRI) [%]
1	Turmeric	5	0.71* (0.00) **	100
		10	0.71 (0.00)	100
		15	0.71 (0.00)	100
2	Garlic	5	0.71 (0.00)	100
		10	0.71 (0.00)	100
		15	0.71 (0.00)	100
3	Absolute control	-	11.62 (86.67)	-
S. Em. ±		0.13		
C. D. a t 5%		0.40		
C.V.%		2.42		

#Average of three repetitions
*Figures outside parenthesis are √x+0.5 transformed value
**Figures in parenthesis are original values

3.4.4 Effect of edible chemicals on penicillium growth *in vitro*
Four edible chemicals-sodium bicarbonate, potassium metabisulphite, sodium chloride, and calcium chloride-were tested at 250, 500, and 750 ppm against *Penicillium digitatum* using the poisoned food technique depicted in Table 3.4. At 250 ppm, potassium metabisulphite showed the highest growth inhibition (93.78%), followed by calcium chloride (91.03%) and sodium bicarbonate (91.00%), while sodium chloride was less effective (58.70%). At 500 ppm,

sodium bicarbonate (94.30%) and potassium metabisulphite (94.22%) were most effective, with sodium chloride at 73.00%. At 750 ppm, all except sodium chloride showed complete growth inhibition.

The growth inhibition increased with higher concentrations.

These results are consistent with previous studies, such as Fallanaj *et al*. (2013) [4], who found sodium bicarbonate effective against *P. digitatum*, and El-Gali (20140) [3], who reported reduced growth with higher calcium chloride concentrations.

Table 6: *In vitro* evaluation of edible chemicals on mycelial growth and growth inhibition of *Penicillium digitatum*

Sr. No.	Name of the Edible chemical	Concentration (ppm)	Average colony diameter(mm)#	Per cent growth Inhibition (%)
1	Sodium bicarbonate	250	2.93* (8.10) **	91.00
		500	2.37 (5.13)	94.30
		750	0.71 (0.00)	100.00
2	Potassium metabisulphite	250	2.47 (5.60)	93.78
		500	2.39 (5.20)	94.22
		750	0.71 (0.00)	100.00
3	Sodium chloride	250	6.14 (37.17)	58.70
		500	4.98 (24.30)	73.00
		750	4.44 (19.23)	78.63
4	Calcium chloride	250	2.93 (8.07)	91.03
		500	2.39 (5.20)	94.22
		750	0.71 (0.00)	100.00
5	Absolute control	-	9.51 (90.00)	-
S. Em. ±		0.14		
C. D. a t 5%		0.42		
C.V.%		1.55		

#Mean of three repetitions
*Figures outside parenthesis are √x+0.5 transformed value
**Figures in parenthesis are original values

3.4.5 Effect of edible chemicals on penicillium growth *in vivo*

Edible chemicals effective *in vitro* were tested *in vivo* on citrus fruits using the fruit dip method. Disease severity data were used to calculate the percent disease index (PDI) and decay reduction index (DRI). All treatments significantly controlled the pathogen compared to the control, showing complete mycelial growth inhibition at 25±1°C depicted in Table 3.5.

Table 7: *In vivo* evaluation of edible chemicals against *P. digitatum*

Sr. No.	Name of the edible chemicals	Concentration (ppm)	Per cent disease index (PDI)#	Decay reduction index (DRI)
1	Sodium bicarbonate	500	0.71* (0.00) **	100
		750	0.71 (0.00)	100
2	Potassium metabisulphite	500	0.71 (0.00)	100
		750	0.71 (0.00)	100
3	Calcium chloride	500	0.71 (0.00)	100
		750	0.71 (0.00)	100
4	Absolute control	-	7.34 (53.33)	-
S. Em. ±		0.15		
C. D. a t 5%		0.46		
C.V.%		3.48		

#Mean of three repetitions
*Figures outside parenthesis are √x+0.5 transformed value
**Figures in parenthesis are original values

Photo 1: Cultural characteristics of *P. digitatum*

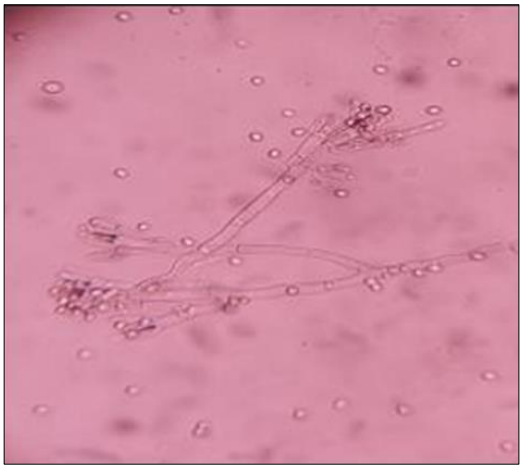

Photo 2: Morphological characteristics of *P. digitatum*

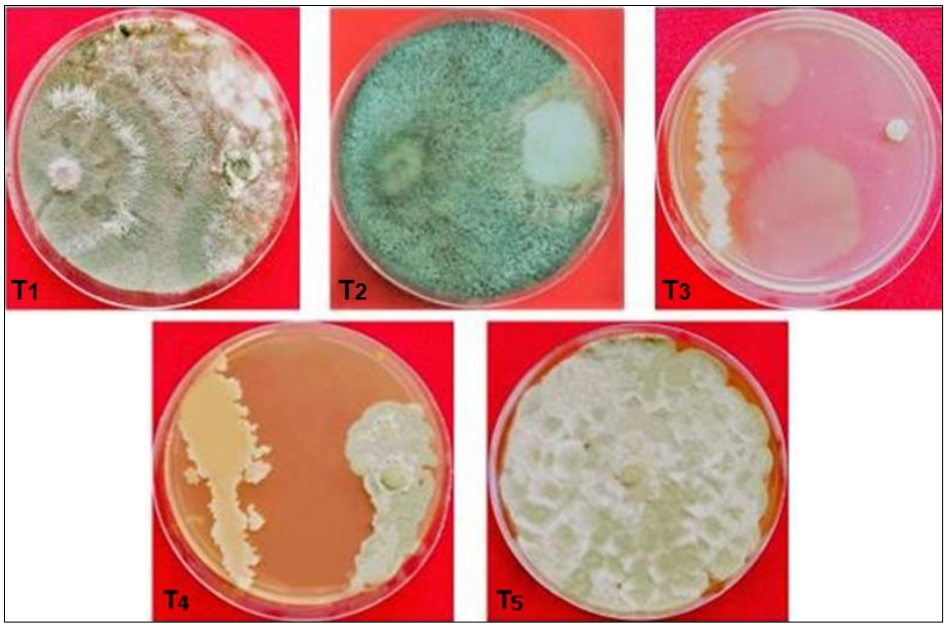

Photo 3: Growth inhibition of *P. digitatum* on PDA with different biocontrol agents *in vitro*

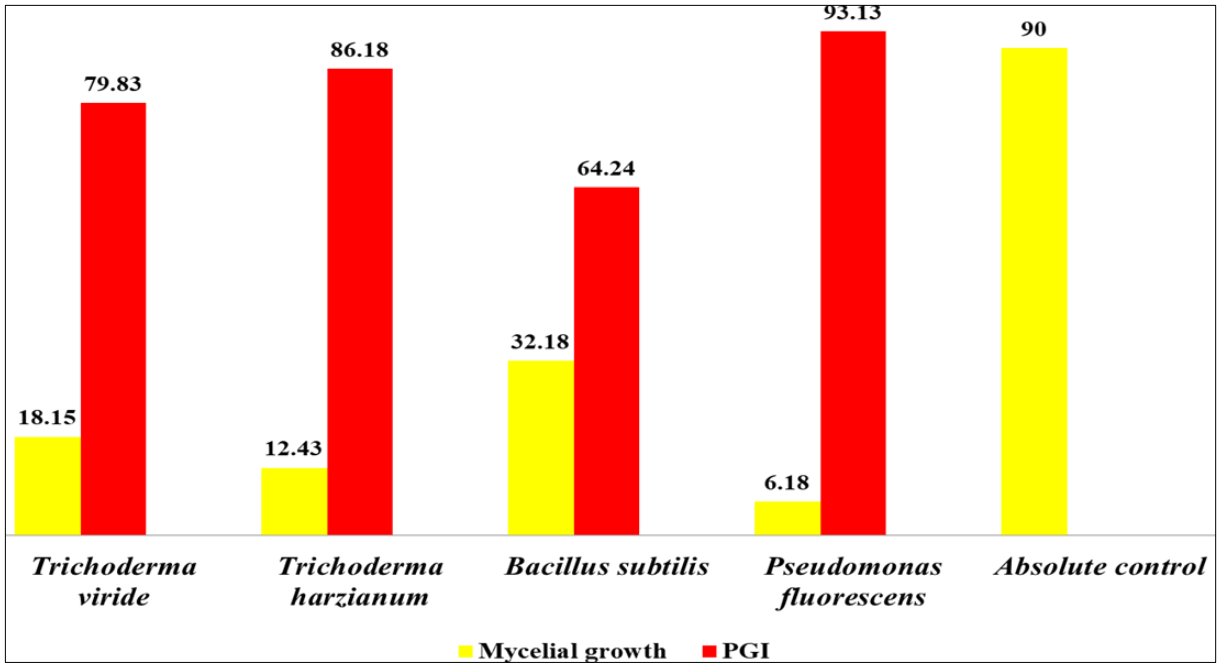

Fig 1: Evaluation of antagonists against *P. digitatum in vitro*

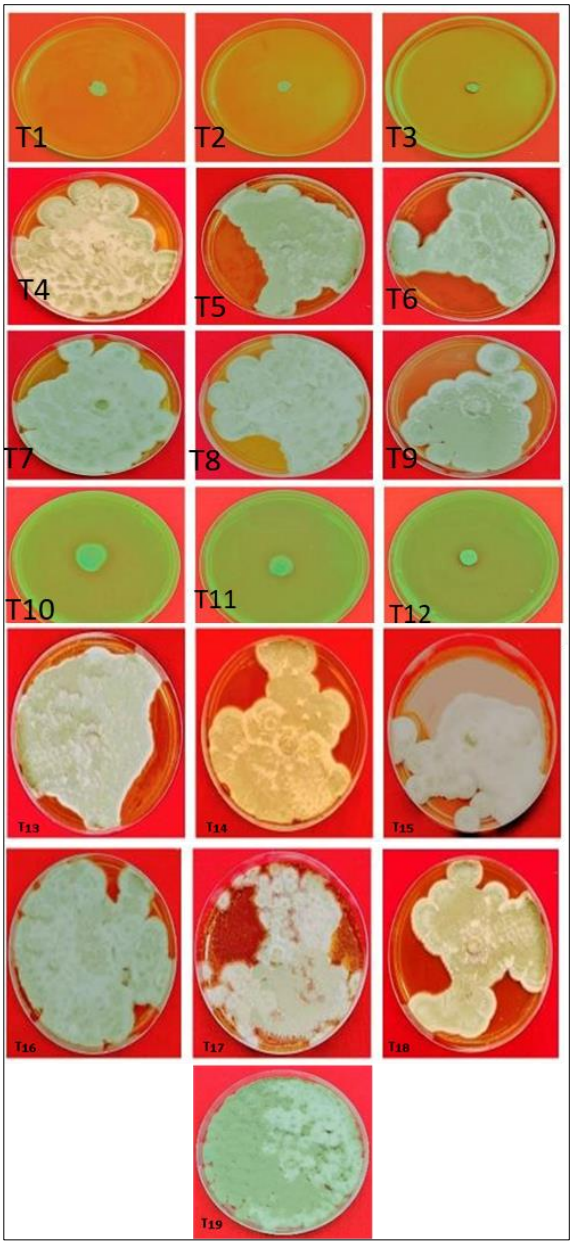

Photo 4: Growth inhibition of *P. digitatum* at different concentrations of plant extracts *in vitro*

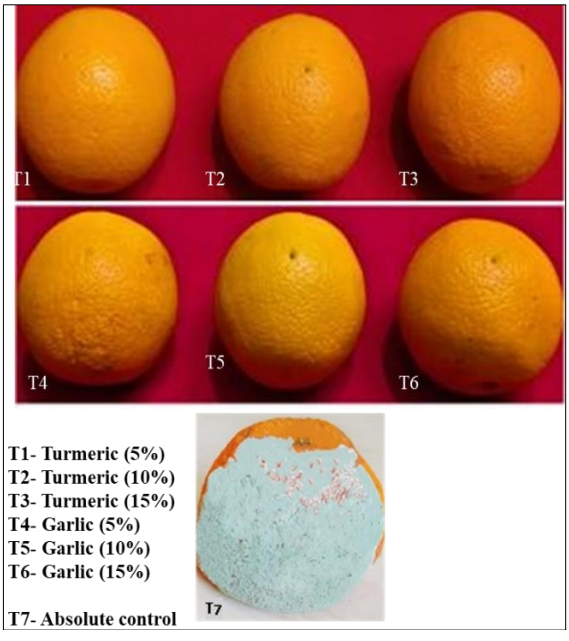

Photo 5: *In vivo* evaluation of plant extracts against *P. digitatum*

4. Summary and conclusion

Citrus, the third most important fruit crop in India, faces severe post-harvest losses due to *Penicillium digitatum* and *P. italicum*, which cause fruit rot during storage and transit. A survey conducted in Navsari markets revealed a disease incidence ranging from 5% to 26.7%, with the highest in Dudia Talav market in February. The pathogen *P. digitatum* was isolated, and its pathogenicity confirmed through Koch's postulates. Cultural studies showed olive-green, fast-growing colonies, while morphological analysis identified hyaline, septate mycelium and green, ellipsoidal conidia. For management, *P. fluorescens* exhibited the highest mycelial growth inhibition (93.13%) among biocontrol agents, followed by *T. harzianum* (86.18%). Turmeric and garlic extracts were highly effective in controlling the pathogen both *in vitro* and *in vivo*. Among edible chemicals, sodium bicarbonate, potassium metabisulphite, and calcium chloride achieved complete inhibition at 750 ppm, while sodium chloride was less effective. These findings suggest that turmeric, garlic, and selected edible chemicals offer sustainable solutions for managing citrus fruit rot effectively.

5. Acknowledgments: Authors are very thankful to the Department of Plant Pathology, Navsari Agricultural University, Navsari, Gujarat, India, for assisting and providing funds and facilities to carry out the research.

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