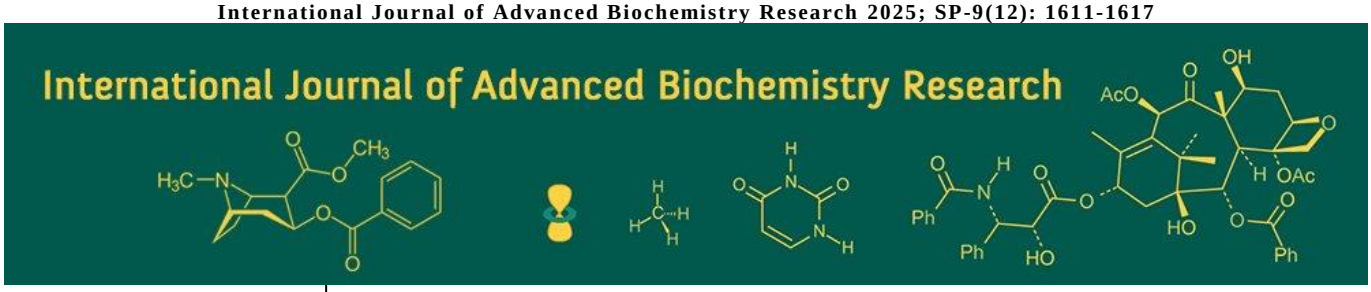

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Integrated management of mango anthracnose using botanicals, natural inputs and fungicides under *in-vitro* and *in-vivo* conditions

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Abstract
Mango suffers post-harvest losses of up to 40%, primarily due to anthracnose caused by *Colletotrichum gloeosporioides*. This study assessed the efficacy of selected plant extracts, natural farming inputs and fungicides against the pathogen under *in-vitro* and *in-vivo* conditions. Garlic (10%) and ginger (10%) extracts showed the highest mycelial inhibition (92.21% and 89.93%), while fresh cow urine (10%) was the most effective natural input (91.38%). Among fungicides, tebuconazole 50% + trifloxystrobin 25% WG at 200 ppm exhibited maximum inhibition (98.47%). In in-vivo tests, garlic and ginger extracts significantly reduced disease severity, with tebuconazole + trifloxystrobin showing the lowest severity (6.02% pre-inoculation; 9.81% post-inoculation). The results highlight the potential of integrating botanicals, natural inputs and modern fungicides for eco-friendly and effective management of mango anthracnose.

Keywords: *In-vivo*, *in-vitro*, plant extract, natural farming input, fungicides

Introduction
Mango (*Mangifera indica* L.), widely known as the "King of Fruits", is one of India's most important tropical crops. Although the country is the world's largest mango producer and a major exporter, post-harvest losses of 25-40% remain a serious challenge. Among these, anthracnose caused by *Colletotrichum gloeosporioides* is the most destructive disease. The pathogen infects mango tissues during flowering and stays latent until fruit ripening, leading to dark, sunken lesions and severe spoilage, ultimately reducing marketability and economic returns.

Conventional control of anthracnose largely depends on synthetic fungicides. While effective, their overuse has resulted in issues such as fungicide resistance, chemical residues, environmental risks and growing consumer health concerns. These drawbacks have encouraged the exploration of safer, eco-friendly alternatives. Botanicals like neem, garlic, tulsi and ginger contain bioactive compounds with strong antifungal activity that can inhibit spore germination and mycelial growth.

Natural farming inputs including cow urine, fermented buttermilk, *jeevamrutha*, *panchagavya* and *sonthastra* are also gaining importance due to their antimicrobial potential and their ability to enhance fruit quality and shelf life. Newer-generation fungicides, particularly triazole-strobilurin combinations, have shown excellent control of *C. gloeosporioides* with reduced residue risks when used judiciously.

Evaluating these treatments under both *in-vitro* and *in-vivo* conditions is crucial for identifying effective and practical disease management options. *In-vitro* studies provide insights into direct antifungal activity, while *in-vivo* trials reflect real post-harvest performance. Therefore, the present study investigates the inhibitory effects of selected plant extracts, natural farming inputs and fungicides against *C. gloeosporioides*, aiming to reduce post-harvest losses and support sustainable anthracnose management in mango.

Material and Methods
The present investigation was carried out at the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, to evaluate the inhibitory effect of various plant extracts, natural farming inputs and fungicides against

C. gloeosporioides, the causal agent of mango anthracnose under *in-vitro* and *in-vivo* conditions.

Evaluation of Plant Extracts under *In-vitro* Conditions

Ten medicinal plants were screened for antifungal activity using the poisoned food technique (Grover and Moore, 1962) [9]. Crude extracts of leaves, roots, or rhizomes were prepared by homogenizing 100 g of fresh plant material in 100 ml of double-distilled water, filtering through muslin cloth. The resulting 100% extract was incorporated into PDA medium at 5% and 10% concentrations (*i.e.*, 2 ml and 4 ml extract in 40 ml PDA, respectively). Twenty milliliters of the amended medium were poured into Petri plates, inoculated with 5 mm mycelial discs of a 7-day-old *C. gloeosporioides* culture and incubated at 27±2 °C. Radial growth and percentage inhibition were recorded using Vincent's formula (1947).

Table 1: List of Plant Extract Treatments

Tr. No.	Treatments	Vernacular name	Plant parts used
T_1-T_2	*Adhatoda vasica* L.	Ardusi	Leaves
T_3-T_4	*Allium sativum* L.	Garlic	Cloves
T_5-T_6	*Azadirachta indica* A. juss	Neem	Leaves
T_7-T_8	*Eucalyptus* spp.	Eucalyptus	Leaves
T_9-T_{10}	*Calotropis procera* A.	Calotropis	Leaves
T_{11}-T_{12}	*Ocimum basilicum* L.	Basil	Leaves
T_{13}-T_{14}	*Vitex negundo* L.	Nagod	Leaves
T_{15}-T_{16}	*Withania somnifera* L.	Ashwagandha	Roots
T_{17}-T_{18}	*Zingiber officinale*	Ginger	Rhizomes
T_{19}-T_{20}	*Mentha arvensis* L.	Mint	Leaves
T_{21}	Control (Test pathogen)	-	-

Evaluation of Natural Farming Inputs under *In-vitro* Conditions

Seven traditional inputs were tested for their antifungal efficacy at 5% and 10% concentrations using the poisoned food technique. Inputs such as cow urine, *sonthastra*, fermented buttermilk, *panchagavya*, *jeevamrutha*, *beejamrutha* and herbal *kunapajala* were either freshly prepared or obtained from reputed sources. Each treatment was mixed into sterilized PDA, poured into Petri plates, inoculated with 5 mm fungal discs and incubated at 27±2 °C. Observations on radial growth and percentage inhibition were recorded.

Table 2: List of Natural Farming Input Treatments

Tr. No	Treatments
T_1-T_2	Cow urine (fresh)
T_3-T_4	*Sonthastra*
T_5-T_6	Sour buttermilk
T_7-T_8	*Panchagavya*
T_9-T_{10}	*Jeevamrutha*
T_{11}-T_{12}	*Beejamrutha*
T_{13}-T_{14}	Herbal *Kunapajala*
T_{15}	Control (Test pathogen)

Evaluation of Fungicides under *In-vitro* Conditions

Nine fungicides in single or combined formulations were evaluated at 100 ppm and 200 ppm concentrations using the poisoned food technique (Nene and Thapliyal, 1993) [11]. Fungicides were incorporated into molten PDA medium and poured into Petri dishes. The plates were inoculated with 5 mm mycelial discs from a 5 day old *C. gloeosporioides* culture and incubated at 27±2 °C. Observations were made on mycelial growth and inhibition.

Table 3: List of Fungicide Treatments

Tr. No.	Treatments
T_1-T_2	Carbendazim 50% WP
T_3-T_4	Azoxystrobin 23% SC
T_5-T_6	Difenoconazole 25% EC
T_7-T_8	Metiram 55% + pyraclostrobin 5% WG
T_9-T_{10}	Tebuconazole 50% + trifloxystrobin 25% WG
T_{11}-T_{12}	Kresoxim-methyl 40% + hexaconazole 8% WG
T_{13}-T_{14}	Azoxystrobin 18.2% + difenoconazole 11.4% w/w SC
T_{15}-T_{16}	Fluxapyroxad 250 g/l + pyraclostrobin 250 g/l SC
T_{17}-T_{18}	Fluopyram 17.7% w/w + tebuconazole 17.7% w/w SC
T_{19}	Control (Untreated check)

All experiments were conducted using a completely randomized design (CRD) with two replications. Growth inhibition was calculated using Vincent's formula (1947):

$$\text{Per cent growth inhibition (PGI)} = \frac{DC - DT}{DC} \times 100$$

The observations recorded during the experiment included radial growth (mm) and growth inhibition (%). Where, DC refers to the mean diameter of the mycelium colony in the control treatment (mm) and DT refers to the mean diameter of the mycelium colony in the treated set (mm).

Evaluation of Plant Extracts and Fungicides under *In-vivo* Conditions

In-vivo Evaluation of Plant Extracts

Five plant extracts (Garlic, Neem, Eucalyptus, Basil and Ginger) were tested at 5% and 10% concentrations. Semi-ripe mango fruits (cv. Dasheri) were surface sterilized with 1% sodium hypochlorite and rinsed with sterile water. Two treatment approaches were followed, Pre-inoculation: Fruits were dipped in treatment solutions for 5 minutes, air-dried and then inoculated by the pin-prick method using a conidial suspension (1×10^5 spores/ml). Fruits were incubated in polythene bags containing moist sterile cotton. Post-inoculation: Fruits were first inoculated and, after 12 hours, treated by dipping in solutions for 5 minutes. Incubation followed the same procedure as above. Disease severity was assessed on the 4th day post-inoculation using a standard 0-5 disease grading scale.

***In-vivo* Evaluation of Fungicides**

Five fungicide formulations were selected based on *in-vitro* efficacy and tested at 100 ppm and 200 ppm concentrations. The same methodology for fruit sterilization, inoculation and incubation was followed as described above for plant extracts. Disease assessment was carried out on day 4 using the same scale.

Grade	Per cent disease on the fruit surface
0	No Disease
1	< 5
2	5.1-10
3	10.1-25
4	25.1-50
5	> 50

$$\text{PDI} = \frac{\text{Sum of the individual disease grades}}{\text{Number of fruits observed} \times \text{Maximum disease grade}} \times 100$$

Results and Discussion

In-vitro Evaluation of Plant Extracts

Among the various treatments, T_7 (Garlic 10%) recorded the highest mycelial growth inhibition (92.21%), showing statistically superior efficacy in suppressing *C. gloeosporioides*. It was found to be at par with T_8 (Ginger 10%), which exhibited 89.93% inhibition, indicating that both garlic and ginger at higher concentrations possess comparable antifungal activity. T_6 (Neem 10%) with 72.25% inhibition emerged as the second-best treatment, showing moderately high effectiveness. On the other hand, lowest growth inhibition was recorded in calotropis with a colony diameter of 66.87 mm at 5 per cent concentration. The result obtained in the present investigation accordance with earlier reports of Hegde *et al.* (2014). They reported that the garlic clove extract (5 and 10 per cent concentration) inhibited the maximum mycelium growth of *C. gloeosporioides*.

Similarly, Asalkar *et al.* (2019) [1], Niazi *et al.* (2022) [10] and Shinde *et al.* (2024) [17] found that garlic clove extract yielded the least mycelium growth and the highest inhibition percentages against different *C. gloeosporioides* at different concentrations.

Table 4: Effect of plant extract on mycelial growth inhibition of *C. gloeosporioides* under *in-vitro* conditions

Tr. No.	Treatments	Conc. (%)	Mycelial growth (mm)	Growth inhibition (%)
T_1	Ardusi (*Adhatoda vasica* L.)	5	53.48	39.94
T_2		10	37.35	58.35
T_3	Garlic (*Allium sativum* L.)	5	12.95	85.94
T_4		10	7.15	92.21
T_5	Neem (*Azadirachta indica* A. Juss.)	5	33.22	63.41
T_6		10	24.73	72.25
T_7	Eucalyptus (*Eucalyptus* spp.)	5	46.88	45.82
T_8		10	30.92	66.5
T_9	Calotropis (*Calotropis procera* A.)	5	66.87	26.75
T_{10}		10	42.19	52.11
T_{11}	Basil (*Ocimum basilicum* L.)	5	42.55	52.67
T_{12}		10	25.48	71.84
T_{13}	Nagod (*Vitex negundo* L.)	5	46.92	47.1
T_{14}		10	32.95	62.56
T_{15}	Ashwagandha (*Withania somnifera* L.)	5	63.86	29.88
T_{16}		10	41.47	53.08
T_{17}	Ginger (*Zingiber officinale* L.)	5	18.01	80.93
T_{18}		10	9.33	89.93
T_{19}	Mint (*Mentha arvensis* L.)	5	47.17	46.57
T_{20}		10	27.02	69.24
T_{21}	Control (Test pathogen)	-	90.00	-
S.Em.±		-	0.88	-
C.D. at 5%		-	3.20	-
C.V. (%)		-	4.02	-

In-vitro Evaluation of Natural Inputs

In the evaluation of natural farming inputs, T_1 (Cow urine 10%) displayed the highest mycelial inhibition (91.38%), proving to be statistically superior. The next best natural farming input was *Sonthastra*, which demonstrated notable antifungal activity with a colony diameter of 16.84 mm and an inhibition percentage of 81.28 per cent at the same concentration. In contrast, the lowest inhibition was observed in Herbal *Kunapajala* at the 5 per cent concentration, where only 40.34 per cent inhibition was recorded, resulting in a relatively larger mycelial growth of 53.69 mm.

These observations are in line with the results reported by previous researchers such as Kulkarni (2009) [7], Kambar *et al.* (2013) [6] and Salam *et al.* (2018) [15], who also found that natural farming inputs were effectively suppress the growth of *C. gloeosporioides* under *in-vitro* conditions. Furthermore, Ashlesha and Paul (2014) [2] and Patel *et al.* (2024) [13] revealed that apart from cow urine, other inputs such as *Panchagavya*, *Jeevamrutha* and *Beejamrutha* have also shown promising results in inhibiting the growth of *Colletotrichum* spp., further validating the findings of the present study.

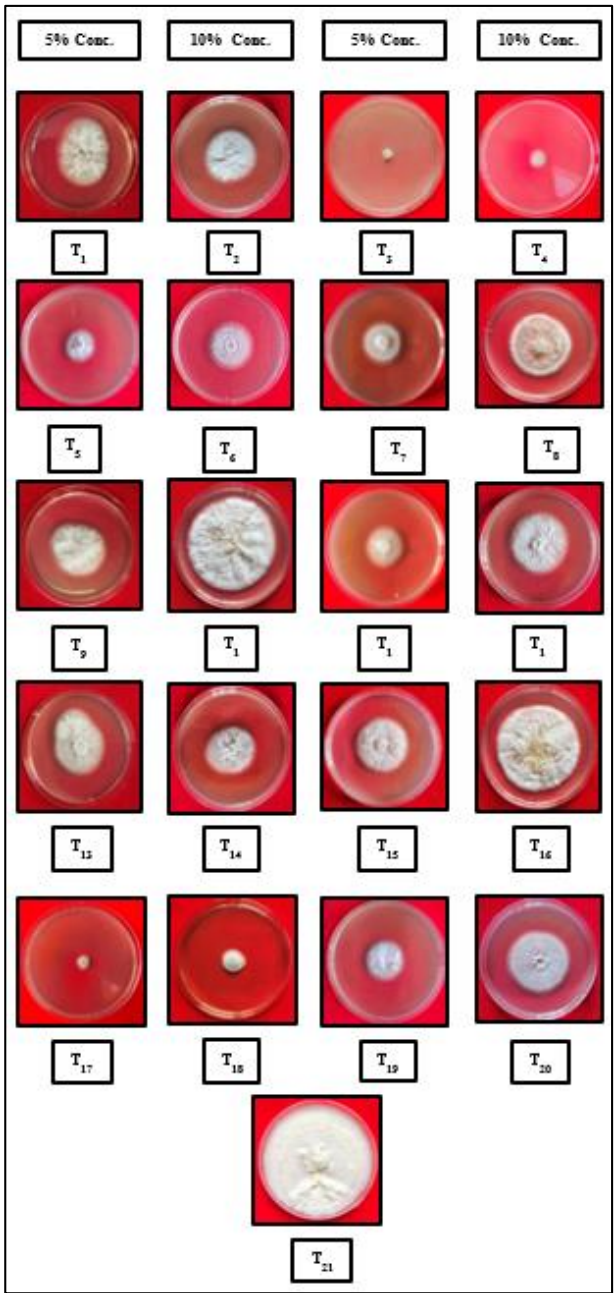

Fig 1: Evaluation of plant extract against *C. gloeosporioides* under *in-vitro* conditions

Table 5: Effect of natural farming input on mycelial growth inhibition of *C. gloeosporioides* under *in-vitro* conditions

Tr. No.	Natural farming inputs	Concentration (%)	Mycelial growth (mm)	Growth inhibition (%)
T_1	Cow urine (Fresh)	5	11.63	87.07
T_2		10	7.75	91.38
T_3	*Sonthastra*	5	22.54	74.95
T_4		10	16.84	81.28
T_5	Sour Buttermilk	5	25.29	71.89
T_6		10	23.14	74.28
T_7	*Panchagavya*	5	26.95	70.05
T_8		10	24.04	73.28
T_9	*Jeevamrutha*	5	43.76	53.31
T_{10}		10	26.34	70.73
T_{11}	*Beejamrutha*	5	48.29	46.33
T_{12}		10	36.24	59.72
T_{13}	Herbal *Kunapajala*	5	53.69	40.34
T_{14}		10	43.16	52.03
T_{15}	Control	-	90.00	-
	S.Em.±	-	0.72	-
	C.D. at 5%	-	2.17	-
	C.V. (%)	-	3.06	-

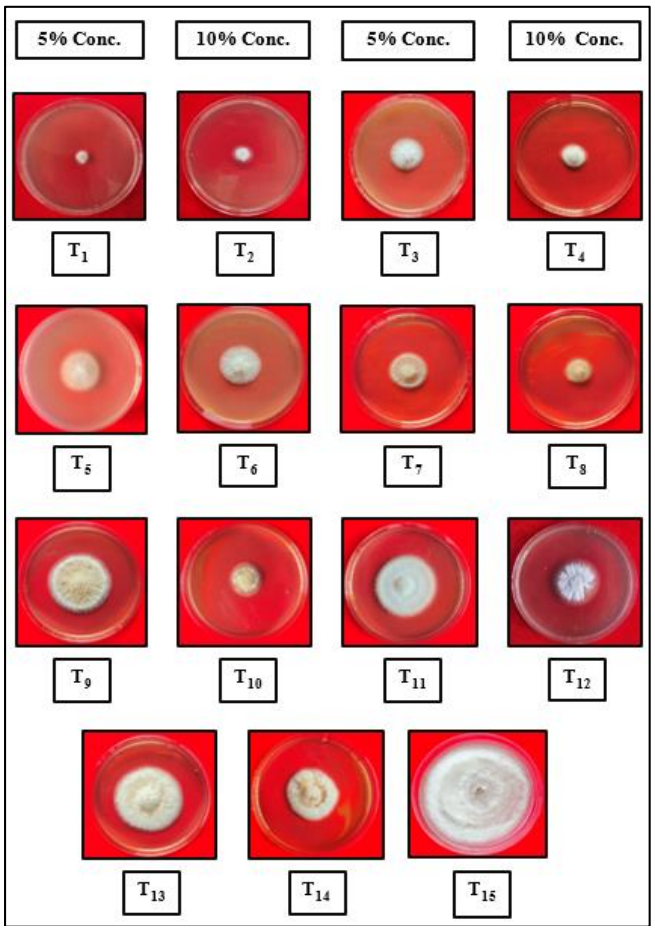

Fig 2: Evaluation of natural farming input against *C. gloeosporioides* under *in-vitro* conditions

In-vitro Evaluation of Fungicides

The fungicide T_6 demonstrated the highest inhibition (98.47%), making it the most effective treatment against the pathogen. It was found statistically at par with T_5. On the other hand, the least effective treatment in suppressing the pathogen was Carbendazim 50% WP at 100 ppm, which recorded the lowest mycelial growth inhibition of only 63.39 per cent, corresponding to a maximum mycelial diameter of 32.94 mm. This indicates that Carbendazim at the lower tested concentration is relatively less effective in managing_*C.gloeosporioides*_under_*invitro*_conditions_compared_to_the_newer_combination_fungicides.

The present findings are in agreement with the results reported by several previous researchers such as Chaudhari and Gohel (2016) [3], Dev and Narendrappa (2016) [4], Kumari *et al.* (2017) [8], Sharma *et al.* (2019) [16], Padghan *et al.* (2023) [12], Ekabote *et al.* (2024) [5] and Raut (2024) [14]. These studies consistently demonstrated that tebuconazole 50% + trifloxystrobin 25% WG and azoxystrobin 18.2% + difenoconazole 11.4% w/w SC are among the most effective fungicide combinations for controlling *Colletotrichum* spp. under laboratory conditions at varying concentrations

Table 6: Effect of fungicides on mycelial growth inhibition of *C. gloeosporioides* under *in-vitro* conditions

Sr. No.	Fungicides	Concentration (ppm)	Mycelial growth (mm)	Growth inhibition (%)
T_1	Carbendazim 50% WP	100	32.94	63.39
T_2		200	22.66	74.81
T_3	Azoxystrobin 23% SC	100	27.43	69.51
T_4		200	17.28	80.8
T_5	Difenoconazole 25% EC	100	28.83	67.96
T_6		200	20.43	77.3
T_7	Metiram 55% + pyraclostrobin 5% WG	100	22.82	74.63
T_8		200	14.98	83.35
T_9	Tebuconazole 50% + Trifloxystrobin 25% WG	100	4.92	94.53
T_{10}		200	1.37	98.47
T_{11}	Kresoxim-methyl 40% + hexaconazole 8% WG	100	19.04	78.83
T_{12}		200	12.94	85.62
T_{13}	Azoxystrobin 18.2% + difenoconazole 11.4% w/w SC	100	6.13	93.18
T_{14}		200	1.72	98.08
T_{15}	Fluxapyroxad 250 g/l + pyraclostrobin 250 g/l SC	100	12.51	86.09
T_{16}		200	8.65	90.38
T_{17}	Fluopyram 17.7 %w/w + tebuconazole 17.7% w/w SC	100	15.32	82.97
T_{18}		200	10.72	88.08
T_{19}	Control (Untreated check)	-	90.00	-
	S.Em.±	-	0.57	-
	C.D. at 5%	-	1.70	-
	C.V. (%)	-	4.17	-

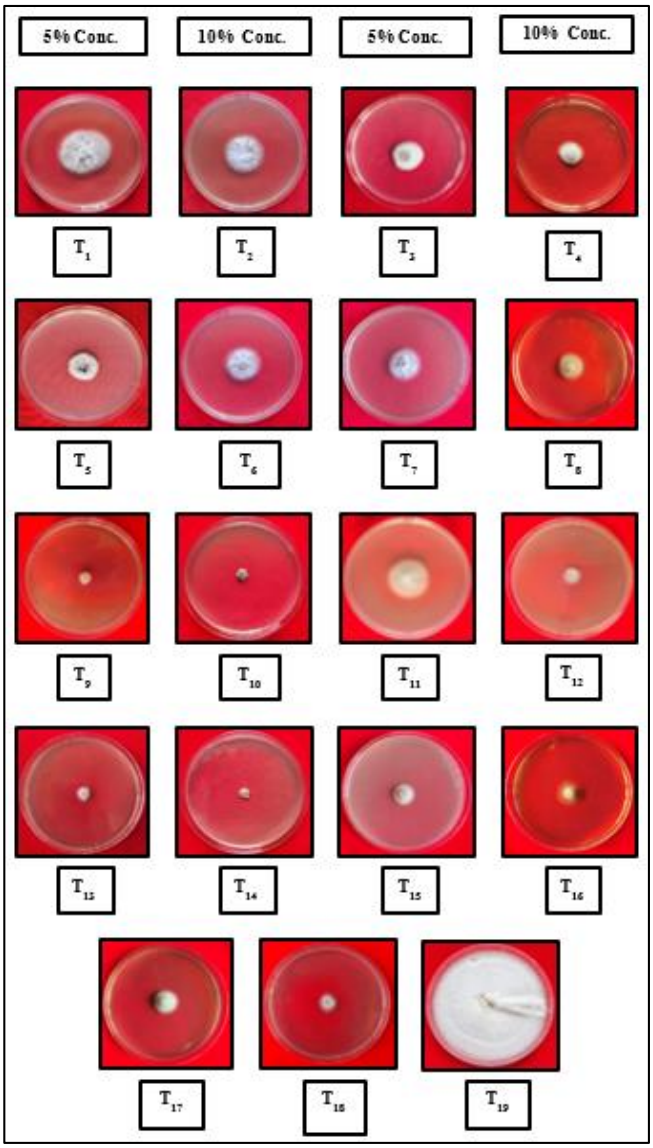

Fig 3: Evaluation of fungicides against *C. gloeosporioides* under *in-vitro* conditions

In-vivo Evaluation of Plant Extracts

Under the pre-inoculation method, garlic clove extract (10%) was the most effective treatment, recording the lowest disease severity of 5.56%. Ginger rhizome extract (10%) was the second most effective, showing 7.41% disease severity. In contrast, eucalyptus leaf extract (5%) was the least effective, with the highest disease severity of 16.67%, indicating its lower antifungal efficacy.

On the 4th day after inoculation, garlic clove extract (10%) continued to show the highest effectiveness, reducing disease severity to 7.41%. Ginger rhizome extract (10%) remained the second most effective, with 11.11% disease severity. Eucalyptus leaf extract (5%) again showed the least efficacy, recording 24.07% disease severity, confirming its limited role in controlling *C. gloeosporioides*.

Table 7: Effect of plant extract on anthracnose caused by *C. gloeosporioides* on mango under *In-vivo* conditions

Tr. No.	Treatments	Disease severity (%)			
		Pre-inoculation		Post-inoculation	
		5%	10%	5%	10%
T_1	Garlic (*Allium sativum* L.)	7.41	5.56	9.26	7.41
T_2	Neem (*Azadirachta indica* A. Juss.)	12.96	9.26	16.67	14.81
T_3	Eucalyptus (*Eucalyptus* spp.)	16.67	14.81	24.07	20.37
T_4	Basil (*Ocimum basilicum* L.)	14.81	12.96	20.37	18.52
T_5	Ginger (*Zingiber officinale* L.)	9.26	7.41	12.96	11.11
T_6	Control (Test pathogen)	18.52	18.52	25.93	24.07
	S.Em.±	0.56	0.50	0.65	0.68
	C. D. at 5%	1.72	1.53	2.01	2.10
	C. V. (%)	7.42	7.58	6.21	7.36

In-vivo Evaluation of Fungicides

Table 8: Effect of fungicides on anthracnose caused by *C. gloeosporioides* on mango under *In-vivo* conditions

Tr. No.	Treatments	Disease severity (%)			
		Pre-inoculation		Post-inoculation	
		100 ppm	200 ppm	100 ppm	200 ppm
T_1	Tebuconazole 50% + trifloxystrobin 25% WG	6.33	6.02	12.71	9.81
T_2	Kresoxim-methyl 40% + hexaconazole 8% WG	18.59	12.01	24.15	20.69
T_3	Azoxystrobin 18.2% + difenoconazole 11.4% w/w SC	11.57	7.54	14.68	12.36
T_4	Fluxapyroxad 250 g/l + pyraclostrobin 250 g/l SC	12.98	9.62	16.53	14.62
T_5	Fluopyram 17.7 %w/w + tebuconazole 17.7% w/w SC	15.78	11.55	20.72	18.91
T_6	Control (Test pathogen)	20.37	18.47	25.66	24.78
	S.Em.±	0.52	0.47	0.66	0.67
	C. D. at 5%	1.59	1.46	2.02	2.08
	C. V. (%)	6.28	7.55	5.95	6.93

On the 4th day after inoculation, the lowest disease severity (6.02%) was observed in fruits treated with tebuconazole 50% + trifloxystrobin 25% WG at 200 ppm, indicating the highest efficacy. This was followed by azoxystrobin 18.2% + difenoconazole 11.4% SC at 200 ppm, which recorded 7.54% disease severity. The least effective treatment was kresoxim-methyl 40% + hexaconazole 8% WG at 100 ppm, with the highest disease severity of 18.59%.

Under post-inoculation conditions, tebuconazole 50% + trifloxystrobin 25% WG at 200 ppm again showed the greatest efficacy, recording the lowest disease severity of 9.81%. Azoxystrobin 18.2% + difenoconazole 11.4% SC at the same concentration was the second most effective, with 12.36% disease severity. Kresoxim-methyl 40% + hexaconazole 8% WG at 100 ppm was the least effective, showing the highest disease severity of 24.15%.

Conclusion

The study conclusively demonstrated that garlic and ginger extracts at 10% concentration possess strong antifungal activity against *C. gloeosporioides*, with garlic emerging as the most effective botanical in both *in-vitro* and *in-vivo* evaluations. Among natural farming inputs, fresh cow urine (10%) exhibited the highest mycelial inhibition, suggesting its potential role in organic disease management practices. New-generation fungicides, especially tebuconazole 50% + trifloxystrobin 25% WG and azoxystrobin 18.2% + difenoconazole 11.4% SC at 200 ppm, provided superior control over the pathogen under both laboratory and fruit application conditions.

Overall, the integration of these effective botanicals, natural inputs and low-residue fungicides offers a promising, sustainable alternative to synthetic chemical-based disease management. This approach not only reduces the dependence on hazardous fungicides but also contributes to improving fruit quality, shelf life and profitability in mango cultivation.

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