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IN-VITRO EVALUATION OF BOTANICALS, ESSENTIAL OILS, BIO-AGENTS AND FUNGICIDES AGAINST *MACROPHOMINA PHASEOLINA* CAUSING DRY ROOT ROT OF SWEET BASIL (*OCIMUM BASILICUM* L.)

Urvashi Singh[1*], Vibha Pandey[1] and Teena Patel[2]
[1]Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), 482004, India
[2]Department of Genetics and Plant Breeding, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), 482004, India
[*]Corresponding author Email: usurvashisingh123@gmail.com
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ABSTRACT

Sweet basil (*Ocimum basilicum* L.) is an economically important medicinal and aromatic crop valued for its essential oil and pharmaceutical properties. However, its productivity is severely constrained by dry root rot caused by *Macrophomina phaseolina*, a destructive soil-borne pathogen. The present study aimed to evaluate the in-vitro efficacy of different plant extracts, essential oils, bio-control agents and fungicides against *M. phaseolina* isolated from sweet basil. Phyto-extracts of *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum* were tested using the poisoned food technique, where neem extract showed maximum inhibition of mycelial growth, while basil extract was least effective. Among essential oils, neem oil exhibited superior antifungal activity compared to tulsi oil, with growth inhibition increasing with concentration. Bio-agents were evaluated using the dual culture technique; among three isolates of *Trichoderma viride* and *Pseudomonas fluorescens*, the sweet basil isolate of *T. viride* showed maximum antagonistic activity followed by the Sarpgandha isolate of it. Chemical fungicides significantly inhibited pathogen growth, with Trifloxystrobin (25%) + Tebuconazole (50% WP) and Copper oxychloride proving most effective. Overall, the study identified highly effective eco-friendly and chemical disease management components under laboratory conditions, providing a scientific basis for selecting suitable treatments for integrated disease management strategies.

Keywords **:** Sweet basil, *Macrophomina phaseolina*, in-vitro evaluation, botanicals, bioagents, fungicides.

Introduction

Sweet basil (*Ocimum basilicum* L.) is an important aromatic and medicinal crop belonging to the family Lamiaceae. It is widely cultivated for its essential oil and pharmaceutical value and is used extensively in perfumery, cosmetics, food flavoring, and traditional medicine systems (Reis *et al.,* 2007). The crop is commercially grown in several parts of India, including Madhya Pradesh, where its cultivation is expanding steadily due to increasing market demand (Patel and Kushwaha, 2013). Despite its economic importance, sweet basil production is severely constrained by soil-borne diseases, among which dry root rot is one of the most destructive.

Dry root rot of sweet basil is caused by *Macrophomina phaseolina* (Tassi, 1901) Goid.,a necrotrophic and opportunistic pathogen with a wide host range. The pathogen has been reported to infect more than 500 plant species worldwide and is widely distributed in tropical and subtropical regions (Das and Indira, 2008). The disease becomes severe under moisture stress and high temperature conditions, leading to significant yield losses (Tesso *et al*., 2005). Infection may occur at any stage of crop growth, beginning from seed germination to maturity, and affects roots, stems, leaves, branches, and inflorescence. Infected plants exhibit longitudinal dark brown to grey lesions on the stem, followed by

defoliation and wilting, eventually resulting in plant death (Reis *et al*., 2007; Abawi and Pastor-Corrales, 1990).

Macrophomina phaseolina is a soil-borne pathogen capable of surviving for long periods through microsclerotia, which persist in soil and plant debris. These structures germinate rapidly under favorable conditions and infect host roots through adhesion and penetration, making disease management difficult (Sinclair, 1989). Due to its broad host range, high survival ability, and lack of resistant cultivars, effective management of dry root rot remains a challenge. Conventional chemical control methods often provide inconsistent results and pose environmental and health concerns.

In recent years, emphasis has been placed on eco-friendly disease management approaches, particularly the use of botanicals, essential oils, bioagents, and other biological alternatives. Botanicals possess antifungal, antibacterial, and antiviral properties and are increasingly explored as sustainable disease management tools (Khaire *et al*., 2018). Plant extracts such as neem, garlic, eucalyptus, and tulsi have shown inhibitory effects against several soil-borne pathogens, including *M. phaseolina* (Sindhan *et al.*, 1999). Their antifungal activity is attributed to the presence of bioactive compounds that interfere with fungal cell wall integrity and metabolic processes.

Induced systemic resistance (ISR) is another important plant defense mechanism that enhances resistance without altering the plant genome. Resistance is mediated through activation of defense enzymes such as peroxidase, chitinase, and polyphenol oxidase, which strengthen host tissues and suppress pathogen colonization (Kuc, 1982; Ye *et al.*, 1990). Abiotic and biotic inducers trigger several defensive mechanisms, such as the deposition of pathogen-related protein in plant tissues (Ye *et al.*, 1990). Bioagents such as *Trichoderma viride* and *Pseudomonas fluorescens* play a significant role in ISR by producing antifungal metabolites, competing for nutrients, and inducing host defense responses (Netu *et al*., 2008; Anand *et al*., 2009).

Considering the limitations of chemical fungicides and the increasing need for sustainable agriculture, evaluation of biological alternatives and newer low-phytotoxic fungicides (Strobilurins and Triazole Group) against soil borne pathogens like *M. phaseolina* becomes essential. Therefore, the present investigation was undertaken to assess the efficacy of different plant extracts, plant oils, bioagents, and fungicides against *Macrophomina phaseolina* causing dry root rot of sweet basil under laboratory conditions.

Materials and Methods

Experimental Location

The present investigation was conducted in the Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya (JNKVV), Jabalpur, Madhya Pradesh, India in 2020. All laboratory experiments were carried out in the Plant Pathology laboratory under controlled conditions.

Laboratory Techniques

Standard laboratory procedures for preparation of culture media, sterilization, isolation, purification, and maintenance of fungal cultures were followed as described by Dhingra and Sinclair (1995), Nene and Thapliyal (1993), and Aneja (2003), with minor modifications wherever required.

Glassware and Cleaning

Borosilicate glassware of standard measurements such as conical flasks, Petri plates, test tubes, pipettes, volumetric flasks, and measuring cylinders were used. All glassware was thoroughly washed with detergent, rinsed with tap water, soaked in cleaning solution (potassium dichromate and concentrated sulphuric acid), rinsed several times with distilled water, and air-dried before use.

Sterilization

Glassware and culture media were sterilized in an autoclave at 121.6°C under 15 psi pressure for 20 minutes. Inoculation tools such as forceps, needles, and cork borers were sterilized by dipping in alcohol followed by flaming. Work was carried out under aseptic conditions in a laminar airflow chamber.

Collection, Isolation, and Maintenance of Pathogen

Diseased sweet basil plants showing root rot symptoms were collected from experimental fields of ACRIP on MAP&B, Department of Plant Physiology, JNKVV, Jabalpur. Diseased root portions were washed thoroughly, cut into small pieces, surface sterilized using 0.1% sodium hypochlorite solution for 30–40 seconds, and rinsed with sterile distilled water. The pieces were aseptically transferred onto Potato Dextrose Agar (PDA) medium and incubated at 25±2°C. Pure cultures of *Macrophomina phaseolina* were obtained through hyphal tip method and maintained on PDA slants at 4°C for further studies.

Culture Media

Potato Dextrose Agar (PDA) was used for isolation and growth of the pathogen. PDA was

prepared by boiling peeled potato pieces, filtering the extract, and adding dextrose and agar. The pH of the medium was adjusted to 6.8 before sterilization.

Evaluation of Phyto-extracts Against *Macrophomina phaseolina* (In-vitro)

Three locally available plant species, viz., *Azadirachta indica* (Neem), *Ocimum basilicum* (sweet basil), and *Eucalyptus globules* were evaluated for their antifungal activity using the poisoned food technique. Fresh leaves were washed, surface cleaned, and homogenized with sterile distilled water in a ratio of 1:1 (w/v). The homogenate was filtered through double-layered muslin cloth to obtain stock solution.

Different concentrations (5, 10, and 15%) of plant extracts were prepared by mixing appropriate quantities of stock solution with molten PDA medium. The poisoned medium was poured into sterilized Petri plates. After solidification, each plate was inoculated with a 5 mm disc cut from the margin of a 7-day-old culture of *M. phaseolina*. Plates without plant extract served as control. Three replications were maintained for each treatment. Plates were incubated at 25±2°C and radial growth was recorded after 96 hours.

Evaluation of Essential Oils

Essential oils of neem and sweet basil were evaluated against *M. phaseolina* under in-vitro conditions. Oils were incorporated into PDA medium at different concentrations using the poisoned food technique. Inoculation, incubation, and observations were recorded following the same procedure as described above.

Evaluation of Bio-agents Against *Macrophomina phaseolina*

Bio-agents including *Trichoderma viride* and *Pseudomonas fluorescens* were evaluated using the dual culture technique. A mycelial disc of *M. phaseolina* was placed on one side of the PDA plate, while the antagonist was placed on the opposite side. Control plates contained only the pathogen. Plates were incubated at 25±2°C and observations on radial growth inhibition were recorded after 96 hours.

Evaluation of Fungicides (In-vitro)

Selected fungicides were evaluated against *M. phaseolina* at different concentrations using the poisoned food technique. Required quantities of fungicides were mixed thoroughly with sterilized molten PDA medium and poured into Petri plates. Inoculation was done using a 5 mm mycelial disc of the test pathogen. Plates were incubated at 25±2°C and radial growth was measured.

Assessment of Antifungal Activity

Percent inhibition of mycelial growth was calculated using Vincent's (1947) formula:

$$\textbf{Percent growth inhibition (\%)} = (C - T) / C \times 100$$

Where:
C = radial growth of the pathogen in control (mm)
T = radial growth of the pathogen in treatment (mm)

Statistical Analysis

All in-vitro experiments were conducted in completely randomized design (CRD). Data obtained were subjected to statistical analysis and mean values were compared at appropriate levels of significance.

Results and Discussion

Isolation and Identification of the Pathogen

The pathogen associated with dry root rot of sweet basil was successfully isolated from infected root samples collected from fields of ACRIP on MAP&B, Department of Plant Physiology, JNKVV, Jabalpur. On PDA medium, the fungus exhibited characteristic mycelial growth. Based on cultural and morphological characteristics, including the formation of microsclerotia, the pathogen was identified as *Macrophomina phaseolina* (Tassi, 1901) Goid.

The disease symptoms observed during the present investigation, such as longitudinal dark brown to grey lesions on roots and basal stem followed by wilting and defoliation, also observed in earlier reports describing dry root rot symptoms caused by *M. phaseolina* (Abawi and Pastor-Corrales, 1990; Reis *et al*., 2007). Similar symptomatology and pathogen identity in sweet basil have also been confirmed by Koike *et al.* (2020), reporting crown and root rot caused by *M. phaseolina*.

Isolation and identification of the pathogen were carried out based on cultural and morphological characteristics. The mycelial and colony features observed in the present study were in agreement with earlier reports by Thirumalachar (1955), Deshpandey *et al.* (1969), and Subhramanyam (1971), who identified the pathogen as *Macrophomina phaseolina*. Further confirmation of the pathogen infecting sweet basil has been reported earlier by Reis *et al*. (2007), and more recently by Koike *et al.* (2020), who reported *Macrophomina* crown rot of sweet basil in the United States. The pure culture of *Macrophomina phaseolina* and the microscopic view of micro-sclerotia produced by the pathogen are shown in Plate 1 and Fig. 1, respectively.

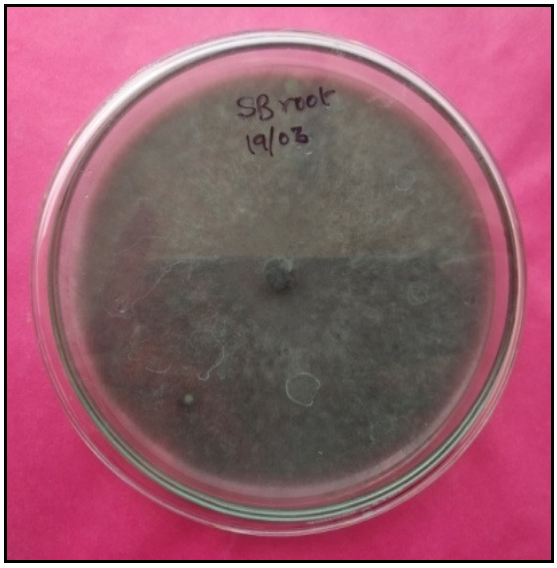

Plate 1 : Pure culture of *Macrophomina phaseolina*

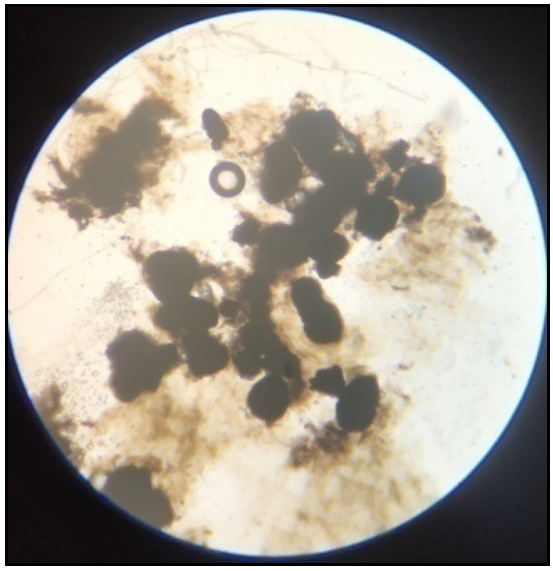

Fig. 1 : Microscopic view of micro-sclerotia produced by the pathogen

1. In-vitro evaluation of phyto-extracts against *M. phaseolina*

The in-vitro efficacy of selected phyto-extracts was evaluated against *Macrophomina phaseolina* using the poisoned food technique. Among the tested phyto-extracts, *Neem* (*Azadirachta indica*) extract showed the maximum inhibition of mycelial growth of the pathogen at all tested concentrations. This was followed by Eucalyptus (*Eucalyptus globulus*) extract, whereas sweet basil (*Ocimum basilicum*) extract recorded the minimum growth inhibition of the pathogen.

The results further indicated that increase in concentration of phyto-extracts led to a corresponding increase in mycelial growth inhibition of *M. phaseolina* under in-vitro conditions. However, none of the tested phyto-extracts resulted in complete inhibition of the pathogen

Table 1: Evaluation of incompatibility of botanical extracts at different concentration with the mycelial growth of the test pathogen

Treatments	Avg. colony dia. mm			Avg.mm (mean factor A)	Avg. inhibition%			Avg. Inhibition % (mean factor A)
	5%	10%	15%		5%	10%	15%	
Neem	53.39	37.71	31.58	40.89	40.67	58.09	64.9	54.55
Eucalyptus	58.5	43.17	37.65	46.44	34.99	52.02	58.15	48.39
Sweet basil	64.62	57.93	53.82	58.79	28.19	35.62	40.19	34.67
Control	90	90	90	90	0	0	0	0
Mean(factor B)	66.62	57.20	53.26		25.96	36.43	40.81	
Factors	C.D.	SE(d)	SE(m)		C.D.	SE(d)	SE(m)	
Factor(A)	0.40	0.19	0.13		0.45	0.21	0.15	
Factor(B)	0.35	0.16	0.11		0.39	0.18	0.13	
Factor(A X B)	0.70	0.33	0.23		0.78	0.37	0.26	

These findings are in close agreement with those of Thombre and Kohire (2018), who evaluated several botanical extracts against *M. phaseolina* and reported higher inhibition with neem-based extracts. Sindhan *et al*. (1999) also reported inhibitory effects of several plant extracts against *M. phaseolina* and *Rhizoctonia solani* at different concentrations, supporting the present observations. The antifungal activity of phyto-extracts may be attributed to the presence of bioactive compounds that interfere with fungal cell wall synthesis and metabolic processes.

2. In-vitro evaluation of essential oils against *M. phaseolina*

The essential oils of *Neem* (*Azadirachta indica*) and sweet basil (*Ocimum basilicum*) were tested against *M. phaseolina* using the poisoned food technique. Among the tested essential oils, Neem oil was found to be significantly more effective, recording the highest percentage inhibition of mycelial growth of the pathogen at all concentrations.

In contrast, sweet basil oil showed comparatively lower inhibition of fungal growth. Similar to phyto-extracts, an increase in essential oil concentration enhanced the inhibitory effect against *M. phaseolina* under in-vitro conditions.

Table 2: Evaluation of incompatibility of botanical oils at different concentration with the mycelial growth of the test pathogen

Treatments	Conc. (ppm)	Mean colony dia. (mm)	Inhibition %
Neem oil (*Azadirachta indica*)	200	32.52	63.85
	250	29.52	67.19
	300	27.77	69.13
Sweet basil oil (*Ocimum basilicum*)	200	80.03	11.06
	250	78.19	13.09
	300	72.44	19.50
Control		90.00	0.00
C.D.		0.34	0.38
SE(m)		0.11	0.12
SE(d)		0.15	0.18
C.V. (%)		0.33	0.63

Similar observations were reported by Kumar *et al*. (2019), who documented the antifungal activity of neem oil against *Rhizoctonia solani* under laboratory conditions. The antifungal efficacy of essential oils may be attributed to the presence of volatile bioactive compounds that disrupt fungal cell membranes, inhibit enzyme activity, and interfere with metabolic pathways.

3. In-vitro evaluation of bio-agents against *M. phaseolina*

The antagonistic efficacy of bio-agents was assessed using the dual culture technique. Three isolates each of *Trichoderma viride* and *Pseudomonas fluorescens*, isolated from Sweet basil, *Sarpagandha*, and *Ashwagandha,* were tested against *M. phaseolina*. Among the fungal bio-agents, the Sweet basil isolate of *Trichoderma viride* exhibited the maximum inhibition of mycelial growth of the pathogen, followed by the Sarpagandha isolate of *T. viride*. The Ashwagandha isolate of *T. viride* showed comparatively lower antagonistic activity. Similarly, among bacterial antagonists, the Sweet basil isolate of *Pseudomonas fluorescens* recorded the highest suppression of mycelial growth, followed by the *Sarpagandha* isolate, whereas the *Ashwagandha* isolate was least effective. Overall, *Trichoderma viride* isolates were more effective than *Pseudomonas fluorescens* in inhibiting the growth of *M. phaseolina* under in-vitro conditions.

Table 3: Evaluation of antagonistic potential of different isolates of bioagents against the test pathogen

Treatments	Mean colony dia. (mm)	Avg. inhibition(%)
T. viride-Sb	19.91	77.69
T. viride- Sg	26.54	70.50
T. viride-Ag	30.92	65.63
P. fluorescens-Sb	28.43	68.40
P. fluorescens-Sg	32.22	64.18
P. fluorescens-Ag	44.57	50.47
Control	90.00	0.00
C.D.	1.05	1.19
SE(m)	0.34	0.39
SE(d)	0.48	0.55
C.V. (%)	1.53	1.19

These results are in agreement with Vinothini *et al*. (2020), who reported significant inhibition of *M. phaseolina* by *Pseudomonas fluorescens*. Jyoti and Saifulla (2017) also reported effective suppression of *M. phaseolina* by *Trichoderma viride*. The antagonistic potential of these bio-agents is attributed to mechanisms such as competition for nutrients and space, mycoparasitism, antibiosis, and secretion of antifungal metabolites.

4. In-vitro evaluation of fungicides against *M. phaseolina*

The efficacy of selected fungicides was evaluated against *M. phaseolina* using the poisoned food technique at different concentrations. Among the tested fungicides, Trifloxystrobin (25%) + Tebuconazole (50% WP) recorded the maximum inhibition of mycelial growth**,** followed by Copper oxychloride (50% WP)**.**

The minimum growth inhibition was observed with Difenoconazole (25% EC)**.** The results also revealed that increasing fungicide concentration significantly enhanced mycelial growth inhibition of the pathogen under in-vitro condition

Table 4: *In vitro* evaluation of mycelial growth suppressing potential of different fungicides against test pathogen

Treatments	Conc. (ppm)	Mean colonydia. (mm)	Inhibition %
Trifloxystrobin 25%+ tebuconazole 50%	500	17.21	80.86
	1000	14.36	84.03
	1500	10.93	87.81
Copper oxychloride 50%	2000	26.86	70.14
	2500	23.36	74.03
	3000	21.41	76.20
Difenconazole 25%	500	34.89	61.22
	1000	32.71	63.64
	1500	26.30	70.76
Control		90.00	0.00
C.D.		0.53	0.59
SE(m)		0.18	0.20
SE(d)		0.25	0.28
C.V.		1.04	0.51

In-vitro evaluation of fungicides revealed that Trifloxystrobin and Tebuconazole were most effective in inhibiting the mycelial growth of *M. phaseolina*. These findings are consistent with earlier reports by Chaudhary (2017), and Parmar *et al*. (2017), who reported high efficacy of these fungicides against soil-borne pathogens. Jebaraj *et al*. (2012) and Khamari and Patra (2018) also documented strong inhibitory effects of copper oxychloride and triazole fungicides against *M. phaseolina*.

Thus, the present investigation clearly demonstrates that botanicals, essential oils, bioagents, and selected fungicides are effective in suppressing *M. phaseolina* under in-vitro conditions, offering eco-friendly and sustainable alternatives for disease management.

Conclusion

The present investigation confirmed *Macrophomina phaseolina* as the causal agent of dry root rot of sweet basil based on cultural and morphological characteristics. In-vitro evaluation of biological, botanical, and new-generation chemical alternatives revealed marked variability in antifungal efficacy. Among phyto-extracts, neem extract showed maximum inhibition of mycelial growth, followed by eucalyptus, while *Ocimum basilicum* extract was least effective. Essential oils were more effective than crude extracts, with neem oil outperforming sweet basil oil and higher concentrations enhancing inhibition. Fungal bio-agents were superior to bacterial antagonists, with the sweet basil isolate of *Trichoderma viride* being most effective. Among fungicides, Trifloxystrobin + Tebuconazole showed highest inhibition, indicating strong potential for integrated disease management.

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