E-ISSN: 2618-0618
P-ISSN: 2618-060X

www.agronomyjournals.com
2024; SP-7(9): 867-869
Received: 02-07-2024
Accepted: 08-08-2024

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In-vitro evaluation of medicinal plant extracts against Fusarium yellows of French bean (*Phaseolus vulgaris* L.) caused by *Fusarium oxysporum* f. sp. *phaseoli*

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DOI: https://doi.org/10.33545/2618060X.2024.v7.i9Sl.1623

Abstract
The recent, there has been a significant increase in the incidence of Fusarium yellows in French beans caused by *Fusarium oxysporum* in the growing areas of Tamil Nadu. The antifungal effects of extracts from six medicinal plants-*Aloe vera*, Tulasi, Omavalli (*Coleus aromaticus*), Nochi, Chitharatthai, and Nithyakalyani-were tested at concentrations of 5% and 10% against *Fusarium oxysporum* f.sp. phaseoli *in vitro*. Among these plants, the 10% concentration of Omavalli (*Coleus aromaticus*) showed the highest efficacy in inhibiting the growth of F.o. f.sp. phaseoli, with a maximum reduction in mycelial growth of 58.04% and 70.11% compared to the control. Conversely, Nithyakalyani (*Vinca rosea*) at 10% concentration was the least effective in suppressing the mycelial growth of *Fusarium oxysporum* f.sp. phaseoli.

Keywords: Fusarium yellows, medicinal plants, fungitoxic, *Fusarium oxysporum*

Introduction
French bean (*Phaseolus vulgaris*) stands out as a crucial leguminous vegetable crop, globally cultivated for its edible beans, including tender vegetables, shelled green beans, and dry beans. It belongs to the legume family Fabaceae and has its origins in Central and Southern America (Bernal and Graham, 2001) [2].

French beans, also known as string beans, field beans, flageolet beans, garden beans, and snap beans, play a vital role in global nutrition, second only to grains in providing calories and protein to populations worldwide. They are recognized for various therapeutic benefits, such as managing diabetes, reducing edema, treating sciatica and chronic rheumatism, alleviating kidney and bladder issues, and reducing uric acid accumulation and albumin loss during pregnancy. Historically, bean leaves have been used to trap bedbugs. In India, French beans are extensively cultivated in states such as Andhra Pradesh, Jharkhand, Maharashtra, Karnataka, Odisha, Uttarakhand, and Tamil Nadu.

During 2019-2020, India cultivated vegetables on approximately 9, 068.30 lakh hectares, yielding about 1, 59, 511.29 lakh tonnes. Beans covered approximately 1, 25.12 lakh hectares, producing about 1,292.33 lakh tonnes. Tamil Nadu alone cultivated French beans on about 4.75 lakh hectares, yielding approximately 95.72 lakh tonnes (www.nbh.com).

French beans are susceptible to various diseases caused by fungal, bacterial, and viral pathogens, posing significant challenges to crop cultivation. Fusarium yellows or wilt, caused by *Fusarium oxysporum* f. sp. phaseoli, is particularly detrimental under conditions of high temperatures and drought (Buruchara and Camacho, 2000) [3]. This vascular disease of beans (*Phaseolus vulgaris* L.) was first observed in the USA in 1929 and later identified as bean yellows, associated with *Fusarium oxysporum* (Schlecht) f.sp. phaseoli (Harter, 1929) [4].

Given concerns about pesticide resistance and environmental impact, scientists are exploring new antimicrobial agents from safe sources like medicinal plants. These plants are known for their antimicrobial and antioxidant properties, with various studies exploring their potential in pathogen control (Tomova *et al*., 2005; Wojdylo *et al*., 2007) [10, 13]. Obongoya *et al*. (2009) [7] investigated the efficacy of *Azadirachta indica*, *Nicotiana tabacum*, and *Vinca rosea* in

controlling *Fusarium oxysporum* Schl. f. sp. phaseoli.

Materials and Methods

The study was conducted in Laboratory of Department of Plant Pathology, Kalasalingam school of Agriculture and Horticulture. The main focus was to isolate the causal organism of fusarium yellows and its management by using plant extracts in poison food technique.

Isolation of the pathogen

The pathogen causing wilt in French beans was isolated from infected samples using the tissue segment method. Infected tissue pieces were first surface sterilized with 0.1% mercuric chloride and rinsed three times with sterile water. These sterilized tissues were then placed onto potato dextrose agar (PDA) in sterile Petri plates and incubated at room temperature (28±2 °C) for 12 days. The fungus was subsequently purified through single spore isolation, and the purified isolates were maintained on PDA slants. Colonies of the fungi were regularly observed under a stereomicroscope and identified using a compound microscope at 40X magnification, following identification keys described by Nelson *et al*. (1983) [6].

Collection medicinal plant extracts

The efficacy of the medicinal plant extracts listed in table 1 were tested against *F.o*. f.sp. *phaseoli*.

Table 1: Medicinal plant extracts screened against *F.o*.f.sp. *phaseoli in vitro*

S. No.	Common name	Scientific name	Parts used	Botanical families
1.	*Aloe vera*	*Aloe vera*	Leaves	Xanthorrhoeaceae
2.	Tulasi	*Ocimum sanctum*	Leaves	Labiatae
3.	Nochi	*Vitex negundo*	Leaves	Verbenaceae
4.	Omavalli	*Coleus aromaticus*	Leaves	Labiatae
5.	Chitharathai	*Alpinia galangal*	Leaves / rhizome	Zingiberaceae
6.	Nithyakalyani	*Vinca rosea*	Leaves/Flower	Apocynaceae

Preparation of medicinal plant extracts (Ansari, 1995)

Fresh plant material (leaves) was collected and washed thoroughly with fresh water. The leaves were ground with sterile water at a ratio of one milliliter per gram of material using a pestle and mortar. The resulting macerate was squeezed through cotton wool to extract the liquid. The extract was then filtered through muslin cloth, followed by Whatman No.1 filter paper, and finally through a Seitz filter to eliminate bacterial contaminants. This process yielded the standard medicinal plant extract solution at 100% concentration. Subsequently, this aqueous extract was diluted to the desired concentration by adding sterilized distilled water as needed.

Efficacy of medicinal plants extracts against the growth of *F.o.* f.sp. *phaseoli in vitro* (Poisoned food technique – Schmitz, 1930)

Ten milliliters (ml) and five ml of the medicinal plant extract were added to sterilized and warm potato dextrose agar (PDA) medium and thoroughly mixed just before plating to achieve concentrations of 10% and 5% of the medicinal plant extract respectively. Twenty ml of this mixture was immediately poured into each sterilized Petri plate and allowed to solidify. A 9 mm culture disc of *Fusarium oxysporum* f.sp. phaseoli (F.o. f.sp. phaseoli) from a well-grown PDA culture was removed using a sterilized cork borer and placed onto the center of each medium plate. All procedures were conducted under aseptic conditions. The plates were then incubated at room temperature (28±2 °C) for a period of 12 days. Potato dextrose agar medium without any plant extract served as the control.

To evaluate the inhibitory effect on fungal growth, the radial growth of the colony was measured in both the control and treatment plates after the incubation period. The percentage inhibition of fungal growth was calculated using the formula:

$$\text{Percentage inhibition } I = 100 \times (C - T)/C$$

Where I is percentage inhibition (mm), C is growth of fungus in the control and T is growth of fungus in the treatment.

Results and Discussion

Among the extracts from various medicinal plants tested, those of *Coleus aromaticus* (Omavalli) (5%) and *Aloe vera* (5%) proved effective, exhibiting significantly reduced mycelial growth-3.73 cm and 4.54 cm, respectively-at 12 DAI. This corresponded to a 58.04% and 48.93% reduction in mycelial growth compared to the control. Following closely were extracts of *Ocimum sanctum* L. (Tulsai) (5%) and Vitex negundo (Nochi) (5%), which showed mycelial growth reductions of 6.12 cm and 6.96 cm, respectively, translating to 31.15% and 21.70% reductions, respectively, and these two were statistically different from each other. *Vinca rosea* (Nithyakalyani) (5%) extract recorded the least reduction in mycelial growth, at 7.87 cm (11.47%) compared to the control (Table 2).

In vitro screening of 10% extracts from different medicinal plants against *Fusarium oxysporum* f. sp. phaseoli showed that the leaf extract of *Coleus aromaticus* (Omavalli) (10%) was most effective, with a 70.11% reduction in mycelial growth over the control at 12 DAI. This was followed by *Aloe vera* (10%) leaf extract, which recorded a 64.66% reduction. *Ocimum sanctum* L. (Tulsai) (10%) and Vitex negundo (Nochi) (10%) leaf extracts ranked next, with reductions of 39.44% and 31.88% over the control, respectively. *Vinca rosea* (Nithyakalyani) (10%) extract showed the least reduction, at 16.11% over the control (Table 2).

The present study indicates that C. aromaticus inhibits the growth of F. oxysporum at both higher and lower concentrations. According to Okigbo and Nmeka (2005) [8], plant and tuber crop diseases can be effectively controlled using plant extracts. Vipin Prakash *et al*. (2005) [12] reported on the antifungal potential of two phytoextracts (Phytoextract-I black colored and Phytoextract-II white colored) from *Withania somnifera* (Ashwagandha) against five phytopathogenic fungi, noting Phytoextract-II's strong inhibition of *Helminthosporium* spp. Karthikeyan and Chandrasekaran (2007) [5] found that plant extracts such as Odiyana wodier, Lawsonia alba, *Ocimum sanctum*, and *Pongamia glabra* effectively reduced mycelial growth (70% to 85% inhibition) and sclerotial germination of Rhizoctonia solani under *in vitro* conditions. Extracts from Avicenia officinales, Cleome viscose, Grewia arborea, Hyptis sueolences, *Ocimum sanctum*, Peltophorum pterophorus, Sesbanian grandiflora, Terminalia chebula, Tephrosia pumila, Tephrosia villosa, and *Withania somnifera* showed high activity,

with Terminalia chebula exhibiting strong antifungal activity against Macrophomina phaseolina even at low concentrations (Varaprasad *et al*., 2009) [11].

Table 2: Effect of extracts of medicinal plants (5%) and (10%) on the mycelial growth of *Fusarium oxysporum* f. sp. *phaseoli in vitro*

S. No.	Common name	Scientific name	Mycelial growth at 12 DAI (cm)*		Growth reduction over control (%)	
			Conc (5%)	Conc (10%)	Conc (5%)	Conc (10%)
1.	*Aloe vera*	*Aloe vera*	4.54	2.97	48.93	67.00
2.	Tulasi	*Ocimum sanctum L.*	6.12	3.26	31.15	63.77
3.	Omavalli	*Coleus aromaticus*	3.73	6.88	58.04	23.55
4.	Nochi	*Vitex negundo*	6.96	3.90	21.70	56.66
5.	Chitharatthai	*Alpinia galanga*	7.28	4.25	18.11	52.77
6.	Nithyakalyani	*Vinca rosea*	7.87	7.87	11.47	12.55
7.	Control	-	9.00	9.00	-	-
CD (P= 0.05)			0.28	0.24		

*Mean of three replications DAI – Days after inoculation

Conclusion

Despite the effectiveness of plant products as substitutes for synthetic fungicides and their proven track record, their widespread practical application remains limited due to farmers' reluctance to adopt natural biofungicides and the lack of extensive research in this area. Additionally, the development of pathogen resistance against fungicides, environmental pollution, and the residual harmful effects of fungicides on non-target organisms are significant drawbacks to their use. Many plants across the globe possess fungicidal, insecticidal, and nematicidal properties. It is imperative to identify their principal and active ingredients and harness them for the benefit of humanity.

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