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BIOLOGICAL AND CHEMICAL CONTROL OF *ASPERGILLUS NIGER* IN SWEET ORANGE: AN *IN VITRO* APPROACH

Vaishnavi P. Kharbadkar[1]*, S.V. Kolase[1], S.B. Latake[1], M.H. Shete[2], A.A. Kale[3] and M.R. Patil[4]

[1]Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India – 413722.

[2]All India Coordinated Research Project on Fruits, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

[3]Department of Biochemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

[4]Department of Statistics, Mahatma Phule Krishi Vidyapeeth, Rahurizx, Maharashtra, India

*Corresponding author E-mail: vkharbadkar@gmail.com

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ABSTRACT

Sweet orange (*Citrus sinensis*) is a nutritionally rich and commercially important fruit crop, but its productivity is severely constrained by fruit rot caused by *Aspergillus niger*, a major fungal pathogen. The present study was undertaken to evaluate the *in vitro* efficacy of selected fungicides and bio-control agents against *A. niger* with the aim of developing an integrated and sustainable disease management strategy. Laboratory experiments were carried out using poisoned food and dual culture techniques under a completely randomized design with three replications. Among the bio-control agents tested, *Trichoderma harzianum* exhibited the highest inhibition of *A. niger* (94.86%), whereas *Pseudomonas fluorescens* and *Bacillus subtilis* were the least effective. Among the fungicides, tebuconazole 50% + trifloxystrobin 25% WG (0.1%) was the most effective, recording 98.64% inhibition, and was statistically at par with chlorothalonil 40% w/w + difenoconazole 4% SC (97.77%). In contrast, Thiophanate methyl proved least effective, with 68.89% inhibition.

Key words: Bio-agents, fungicides, *Aspergillus niger*, sweet orange.

Introduction

Fruits are rich sources of essential vitamins and minerals and play a vital role in reducing deficiencies of vitamin C and vitamin A. Among them, citrus fruits are highly valued for their excellent organoleptic qualities, palatability, and rich nutritional profile, which includes vitamins, minerals, and antioxidants. *Citrus sinensis* (sweet orange) is an important horticultural commodity in global fruit markets, ranking next to *Vitis vinifera* (grape), largely due to its superior postharvest quality and extended shelf life.

However, citrus production is severely constrained by biotic factors, which are estimated to cause more than 35% fruit losses during both preharvest and postharvest stages, primarily due to phytopathogenic organisms that induce fruit rot. Postharvest rot alone poses a major challenge during citrus storage and transportation, accounting for 20–30% spoilage, and in severe cases reaching up to 50%, resulting in substantial economic damage (Salunkhe *et al.*, 1991). Fungal pathogens, bacterial agents, and viral infections have been identified as the primary biotic threats to citrus crop health and productivity (Singh and Jain, 2004), with fungal pathogens being particularly destructive in causing fruit rot.

Among the fungal pathogens, *Aspergillus niger* is recognised as one of the most devastating agents responsible for fruit rot in sweet orange, affecting fruits at both preharvest and postharvest stages. The frequent occurrence of this pathogen, along with the associated quantitative and qualitative losses, highlights the urgent need for effective management strategies. In this context, the present study was undertaken to identify reliable measures for the management of *A. niger* through an integrated approach involving chemical fungicides and beneficial bio-control agents. Adoption of such integrated disease management practices can reduce dependence on chemical fungicides, promote sustainable and eco-friendly disease control, and ultimately contribute to minimizing postharvest losses while improving crop health and productivity in sweet orange cultivation.

Material and Methods

All laboratory experiments for the present investigation were conducted in the Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri. To evaluate the *in vitro* efficacy of fungicides and bio-agents against the causal fungus, fungicides belonging to different chemical groups were procured from the Department of Plant Pathology and Agricultural Microbiology. These fungicides were stored at 25°C in the dark to preserve their biocidal activity. The fungicides tested in this study were: carbendazim 12% + mancozeb 63% WP (0.15%), tebuconazole 50% + trifloxystrobin 25% WG (0.1%), chlorothalonil 40% w/w + difenoconazole 4% w/w SC (0.1%), azoxystrobin 18.2% + difenoconazole 11.4% w/w SC (0.1%), thiophanate methyl 70% WP (0.1%), and metalaxyl 4% + mancozeb 64% WP (0.2%). The efficacy of these fungicides against *A. niger* was assessed using the poisoned food technique (Nene and Thapliyal, 1993) in a Completely Randomized Design with three replications.

Pure cultures of bio-control agents, namely *Trichoderma harzianum*, *T. koningii*, *T. viride*, *T. virens*, *T. asperellum*, *Bacillus subtilis*, and *Pseudomonas fluorescens*, were obtained from the Department of Plant Pathology and Microbiology, PGI, MPKV, Rahuri, and used in the study. The antagonistic potential of these bio-agents against the causal fungus was tested using the dual culture technique, also laid out in a Completely Randomized Design with three replications.

Plate 1: *In vitro* efficacy of bio-agents against *A. niger*.

Table 1: *In vitro* efficacy of bio-agents against *A. niger*.

Tr. No.	Bio agents	Mean Colony diameter	Per cent Inhibition
T_1	*T. harzianum*	**4.62**	**94.86(76.89)**
T_2	*T. viride*	8.97	90.03(71.59)
T_3	*T. virens*	9.80	89.11(70.73)
T_4	*T. asperellum*	11.65	87.05(68.90)
T_5	*T. koningii*	16.68	81.46(64.49)
T_6	*P. fluorescens*	55.98	37.80(37.93)
T_7	*B. subtilis*	66.84	25.73(30.48)
T_8	Control	90.00	00.00(00.00)
	SEm±	**0.23**	
	CD at 1%	**0.73**	

Observations on radial mycelial growth (colony diameter) of the test pathogen were recorded after seven days of incubation. The efficacy of fungicides and bio-agents was expressed as per cent inhibition of mycelial growth over control, calculated using the formula suggested by Vincent (1927):

$$I = \frac{(C - T)}{C} \times 100$$

Where,

I = Per cent inhibition of mycelial growth (mm)

C = Radial growth of fungus in the control plate (mm)

T = Radial growth of fungus in the treatment plate (mm)

Results and Discussion

In vitro efficacy of bio-agents

Evaluation of bio-agents under *in vitro* conditions clearly demonstrated their effectiveness in suppressing the mycelial growth of *A. niger*, the causal organism of fruit rot in sweet orange (Table 1, Plate 1, Fig. 1). Among all the bio-agent treatments, *T. harzianum* proved to be the most effective, recording the highest inhibition (94.86%). This was followed by *T. viride* (90.03%), *T. virens* (89.11%), *T. hamatum* (87.05%), and *T. koningii*

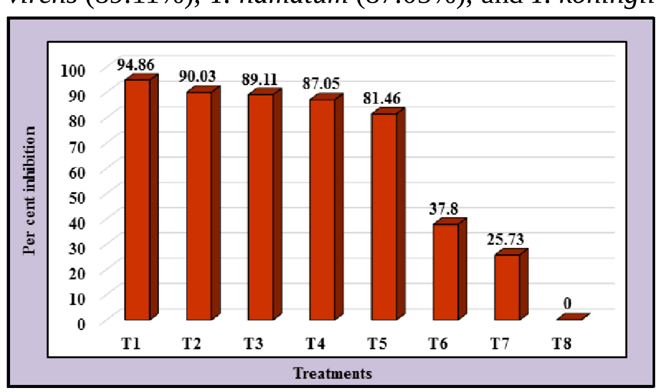

Fig. 1: *In vitro* efficacy of bio-agents against *A. niger*.

Table 2: *In vitro* efficacy of fungicides against *A. niger.*

Tr No.	Name of fungicides	Conc.(%)	Mean colony diameter (mm)	Per cent Inhibition
T_1	Carbendazim 12%+ Mancozeb 63% WP	0.15%	7.87	91.26(72.80)
T_2	Tebuconazole 50% + Trifloxystrobin 25% WG	0.1%	**1.23**	**98.64(83.30)**
T_3	Chlorothalonil 40% w/w + Difenconazole 4.0 SC	0.1%	2.00	97.77(81.41)
T_4	Azoxystrobin 18.2% + Difenconazole 11.4% SC	0.1%	4.23	95.30(77.47)
T_5	Thiophanate methyl 70 WP	0.1%	28.00	68.89(56.09)
T_6	Metalaxyl 4%+ Mancozeb 64% WP	0.2%	15.06	83.27(65.85)
T_7	Control	-	90.00	00.00(00.00)
	SEm ±		**0.35**	
	CD at 1%		**1.16**	

(81.46%). In contrast, *P. fluorescens* and *B. subtilis* showed comparatively lower efficacy, with inhibition percentages of 37.80% and 25.73%, respectively.

These results are in close agreement with the findings of Pudake *et al.*, (2019), who reported that *T. harzianum* exhibited maximum antagonistic activity against *A. niger* with 99.93% mycelial growth inhibition, followed by *T. viride* (98.75%), *T. koningii* (88.43%), *T. hamatum* (77.74%), and *T. lignorum* (72.52%). On the other hand, *P. fluorescens* and *G. virens* were relatively less effective, recording 50.59% and 66.76% inhibition, respectively.

Similarly, Sutar *et al*., (2024) observed that among the *Trichoderma* species tested, *T. harzianum* and *T. asperellum* were most effective against *A. niger*. Although *P. fluorescens* and *B. subtilis* also displayed antifungal activity, their efficacy was markedly lower compared to *Trichoderma* species.

In vitro efficacy of fungicides

Evaluation of fungicides under *in vitro* conditions revealed a significant inhibitory effect on the mycelial growth of *A. niger*, the causal organism of fruit rot in sweet orange (Table 2, Plate 2, Fig. 2). Among the treatments, Tebuconazole 50% + Trifloxystrobin 25% WG (0.1%) was the most effective, recording 98.64% inhibition, which was statistically at par with chlorothalonil 40% w/w + difenconazole 4.0 SC (97.77%). This was followed by azoxystrobin 18.2% + difenconazole 11.4% SC (0.1%), carbendazim 12% + mancozeb 63% WP (0.15%), and metalaxyl 4% + mancozeb 64% WP (0.2%), which showed inhibition rates of 95.30%, 91.26%, and 83.27%, respectively. Thiophanate methyl 70 WP (0.1%) proved to be the least effective, with only 68.89% inhibition.

These findings are consistent with the results of Mahajan *et al.,* (2016), who reported that triazole fungicides, particularly propiconazole and hexaconazole, completely inhibited the mycelial growth of *A. niger* under *in vitro* conditions. Similarly, Sutar *et al.,* (2024) evaluated a range of fungicides, including carbendazim 50% wp, tebuconazole 25.9% EC, hexaconazole 5% EC, propiconazole 25% EC, thiophanate methyl 70% WP, fosetyl-Al 80% WP, copper hydroxide 77% WP, copper oxychloride 50% WP, mancozeb 75% WP, chlorothalonil 75% WP, zineb 75% WP, and dinocap 48% EC against *A. niger*.

Their results also highlighted the superiority of triazole fungicides, as tebuconazole 25.9% EC and hexaconazole

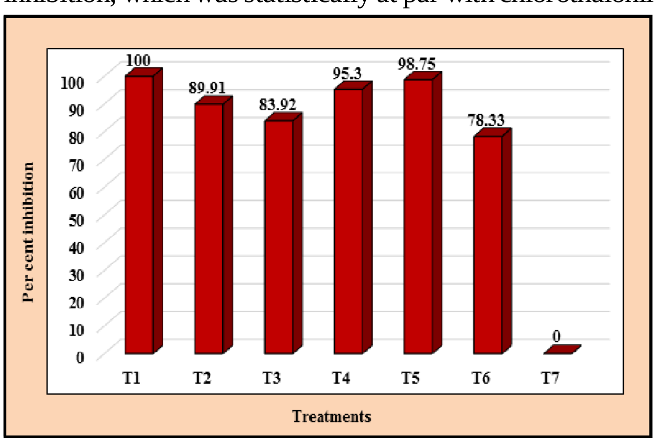

Fig. 2: *In vitro* efficacy of fungicides against *A. niger.*

Plate 2: *In vitro* efficacy of fungicides against *A. niger.*

5% EC achieved complete (100%) inhibition, while thiophanate methyl 70% WP was least effective, with only 59.44% inhibition.

Conclusion

The findings of the present study demonstrated that fruit rot of sweet orange caused by *Aspergillus niger* can be effectively managed through the use of suitable bio-control agents and fungicides. Among the bio-agents tested, *Trichoderma harzianum* and *Trichoderma viride* exhibited strong antagonistic activity against *A. niger* under *in vitro* conditions. Similarly, the fungicidal combinations tebuconazole 50% + trifloxystrobin 25% WG (0.1%) and chlorothalonil 40% w/w + difenoconazole 4.0 SC (0.1%) recorded the highest levels of mycelial inhibition, confirming their potential in managing the pathogen. These findings highlight the importance of integrating bio-agents and effective fungicides in developing eco-friendly and reliable strategies for the management of sweet orange fruit rot.

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