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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
TPI 2021; 10(11): 2830-2833

www.thepharmajournal.com
Received: 28-09-2021
Accepted: 02-11-2021

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Exploring the antifungal potential of bio-agents and phytoextracts against cucumber anthracnose caused by *Colletotrichum orbiculare* (Berkely and Montagne) Von Arx: An *in vitro* investigation

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Abstract
Cucumber (*Cucumis sativus*), known as Khira and Sasha, is a creeping vine extensively cultivated in various seasons across India, particularly in the northern regions and throughout mild tropical areas year-round. In the agricultural landscape of India, cucumber production accounted for 1696 metric tonnes in 2018-19, spanning an area of 109,000 hectares.

This study investigated the efficacy of various bioagents against *Colletotrichum orbiculare*, a prevalent cucumber pathogen. Results demonstrated significant fungistatic/antifungal activities of all evaluated bioagents, notably *Trichoderma harzianum* exhibiting maximum inhibition of mycelial growth (72.60%). Furthermore, botanical extracts, including Neem leaves (*Azadirachta indica*), Ginger rhizome (*Zingiber officinale*), and Garlic bulb (*Allium sativum*), exhibited potent antifungal properties against *C. orbiculare*, with Neem leaves extract displaying the highest inhibition (67.41% at 15% concentration).

Additionally, water extracts from various plants were tested for antifungal properties against *C. lagenarium*, with Garlic bulb extracts showing the highest inhibition (77.73%), followed by *Lawsonia inermis* (72.97%). These findings underscore the potential of bioagents and botanical extracts as eco-friendly alternatives for managing cucumber diseases, aligning with previous research highlighting the efficacy of Garlic extract in inhibiting fungal growth.

Keywords: Cucumber, anthracnose, eco-friendly, bioagents, phytoextracts

Introduction
"Cucurbits", a term coined by Liberty Hyde Bailey for cultivated species of the family *Cucurbitaceae* are a highly evolutionary group of vegetables for human consumption from the remote age of civilization. Cucumber is a creeping vine popularly known as Khira and Sasha (Rahman, 2008) [16] it is cultivated in both *Kharif* and the summer season in the northern part of the country and around the year under mild tropical regions. In India, total cucumber production is 1696 metric tonnes and is cultivated in 109 (000 ha) areas in 2018-19 (FAOSTAT, 2018-19). In Maharashtra, the total area under cucumber cultivation is 4.77 ('000 ha) and production is 62.11 ('000 MT) and contributes 4.73% of the total share of India (FAOSTAT, 2018-19). Cucumber is prone to several fungal, bacterial, and viral diseases which cause serious economic losses to the crop. Mostly fungal diseases which include Downy mildew (*Pseudoperonospora cubensis*); Powdery mildew (*Sphaerotheca fuliginea*; *Erysiphae cichoracearum*); Anthracnose (*Colletotrichum orbiculare* syn. C. *lagenarium*); Cercospora leaf spot (*Cercospora citrullina*); Alternaria leaf spot (*Alternaria alternata*); Damping-off (*Pythium* spp.); Fusarium wilt (*Fusarium oxysporum* f. sp. *cucumerinum*) and Phytophthora crown and root rot (*Phytophthora capsici*) inflict huge economic losses worldwide (Zitter *et al.*, 1998; Saha, 2002) [40, 22]. Of these, Downy mildew, Powdery mildew, Anthracnose, and Alternaria leaf spot are the most widespread and economically important destructive diseases all over the world (Zitter *et al.*, 1998) [40]. In India, powdery mildew, downy mildew, and anthracnose diseases are recognized as serious diseases of the crop (Rai *et al.*, 2008) [19]. *Colletotrichum* spp. is one of the most important plant pathogens in India, causing anthracnose disease in a wide range of hosts including cereals and grasses, legumes, fruits, vegetables, perennial crops, and trees (Rojas *et al.*, 2010) [21]. *Colletotrichum orbiculare* is widely distributed throughout the world.

Materials and Methods

The studies on Anthracnose of Cucumber caused by *Colletotrichum orbiculare* (Berkely and Montage) von Arx experiments were conducted at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

In vitro efficacy of bioagents

Fungal antagonists *viz*., *Trichoderma asperellum*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma hamatum* and *Aspergillus niger*, and two bacterial antagonists *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro* against *Colletotrichum orbiculare*, applying Dual culture technique.

In vitro efficacy of Plant extracts/botanicals

Plant species reported to exhibit antifungal and therapeutic properties against fungal pathogens and available locally were collected from the farms of the College of Agriculture, VNMKV, Parbhani, and adjoining fields. Aqueous extracts of these seven botanicals were evaluated *in vitro* against *Colletotrichum orbiculare* by applying the Poisoned food technique.

Results and Discussions

Present studies on the anthracnose of cucumber caused by *Colletotrichum orbiculare* were undertaken on the aspects *viz*., isolation and identification, pathogenicity test, morphological and cultural characteristics of the test pathogen on culture media, nutritional and physiological requirements, *in vitro* bioefficacy of fungicides, bioagents, and phytoextracts. The results obtained on all these aspects are presented in the following paragraphs.

In vitro evaluation of bioagents/ antagonists

The results obtained on mycelial growth and inhibition of *Colletotrichum orbiculare* with six fungal and two bacterial antagonists are presented in (Table 1 and PLATE-I).

Table 1: *In vitro* efficacy of different bioagents against mycelial growth and inhibition of *Colletotrichum orbiculare*

Tr. No.	Name of Bioagent	Colony Dia.*(mm)	% Inhibition
T_1	*Trichoderma asperallum*	65.46	27.26 (31.47)
T_2	*T. harzianaum*	24.66	72.60 (58.43)
T_3	*T. hamatum*	44.26	50.82 (45.46)
T_4	*T. koningii*	58.10	35.44 (36.53)
T_5	*T. longibrachiatum*	55.93	37.85 (37.96)
T_6	*Aspergillus niger*	30.46	66.15 (54.42)
T_7	*Bacillus subtilis*	58.3	35.22 (36.40)
T_8	*Pseudomonas fluorescens*	57.6	36.00 (36.86)
T_9	Control	90.00	00.00 (00.00)
	SE(m) ±	0.10	0.41
	C.D (P=0.01)	0.30	1.24

*Mean of three replications, Dia: Diameter
Figures in parenthesis are arc sine transformed values.

Results revealed that all the bioagents evaluated exhibited fungistatic/antifungal activity against *Colletotrichum orbiculare* and significantly inhibited its growth over the untreated control. The antagonistic activity of fungal bioagents against *Colletotrichum orbiculare* revealed that all eight antagonistics caused significant inhibition of the mycelial growth of the test fungus (Table 1). However, maximum inhibition of mycelial growth was obtained with *T. harzianum* (72.60%) with least colony diameter 24.66 mm which was significantly superior over all the treatments. This was followed by *Aspergillus niger* (66.15%) and *T. Hamatum* (50.82%) with mycelial growth reduction of 24.66 mm, 30.46 mm, and 44.26 mm respectively.

These were followed by *Trichoderma longibrachiatum* (col. dia.:55.93 mm and inhibition: 37.85 %) and *Trichoderma koningii* (col. dia.: 55.93 mm and inhibition: 37.83 %). The bacterial antagonists *Pseudomonas fluorescens* and *B. substilis* were found to cause significant inhibition of mycelial growth of the test fungus (*Colletotrichum orbiculare*) with 57.6 mm and 58.3 mm linear mycelial growth and 36.00 % and 35.00 % mycelial inhibition, respectively. The least inhibition of mycelial growth was found with *T. aspergillum* (27.26%).

The present results confirmed with Bal and Behera (2012) [4] that *Trichoderma harzianum is* best for reducing the radial growth of test fungus up to (54.91%) followed by *Trichoderma viride* (47. 54%). Among the bacterial bioagents, *Pseudomonas fluorescens* inhibited more than (66%) growth reduction followed by *Bacillus subtilis*.

In vitro evaluation of plant extracts/botanicals

Aqueous extracts of 8 botanicals were evaluated in vitro (each @ 15% and 20 %) against *Colletotrichum orbiculare* and the results obtained on its mycelial growth and inhibition are presented in Table-2 and PLATE II (A and B). Results (Table 2) revealed that all 8 botanical extracts tested were fungistatic/antifungal to *Colletotrichum orbiculare*, which significantly reduced mycelial growth and increased its inhibition over the untreated control. The mycelial growth was found to be decreased and its inhibition was increased with an increase in concentrations of the botanicals tested.

Table 2: *In vitro* efficacy of different botanicals/plant extract against mycelia growth and inhibition of *Colletotrichum orbiculare*

Tr. No.	Treatment	Colony Dia.*(mm)		% Inhibition	
		@15%	@20%	@15%	@20%
T_1	Nirgudi (*Vitex* spp.)	60.93	46.33	32.30 (34.63)	48.52 (44.15)
T_2	Onion (*A. cepa*)	45.70	44.16	49.22 (44.55)	50.93 (45.53)
T_3	Garlic (*A. sativum*)	35.56	5.00	60.48 (51.04)	94.44 (76.36)
T_4	Turmeric (*C. longa*)	47.33	23.30	47.41 (43.51)	74.11 (59.41)
T_5	Tulsi (*O. sanctum*)	56.93	52.56	36.74 (37.31)	41.60 (40.16)
T_6	Ginger (*Z. officinalis*)	29.73	29.33	66.96 (54.91)	67.41 (55.18)
T_7	Neem (*A. indica*)	29.33	22.06	67.41 (55.18)	75.48 (60.31)
T_8	Shatavari (*A. racemosus*)	76.43	61.63	15.07 (22.84)	31.52 (34.15)
T_9	Control	90.00	90.00	00.00 (00.00)	00.00 (00.00)
SE (m) ±		0.821	0.530	0.045	0.040
C.D (P=0.01)		2.457	1.585	0.135	0.12

*Mean of three replications, Dia: Diameter, Figures in parenthesis are arc sine transformed value.

Mycelial Growth and Percent Inhibition

At 15 percent concentration [Table-2 and PLATE-II (A)] radial mycelial growth of the test pathogen (*Colletotrichum*

orbiculare) ranged from 29.33 mm (Neem leaves extract) (*A. indica*) to 76.43 mm Shatavari leaves extract (*A. racemosus*), as against 90.00 mm in untreated control. Least mycelial growth was recorded with Neem leaf extract (*A. indica*) (29.33 mm) which was significantly superior overall treatment and was found at par with Ginger rhizome extract (*Z. officinale*) (29.73 mm), Garlic clove extract (*A. Sativum*) (35.56 mm), Onion bulb extract (*A. cepa*) (45.70 mm), Turmeric rhizome extract (*C. longa*) (47.33 mm), Tulsi leaves extract (*O. Sanctum*) (56.93 mm) and Nirgudi leaves extract (*Vitex* spp.) (60.93 mm). However, maximum mycelial growth was recorded from Shatavari leaves extract (*A. racemosus*) with mycelial growth was 76.43 mm.

Maximum mycelial inhibition at 15 percent concentration was 67.41% with Neem leaf extract (*A. indica*) followed by Ginger rhizome extract (*Z. officinealis*) (66.96%), Garlic bulb extract (*A. sativum*) (60.48%), Onion bulb extract (*A. cepa*) (49.22%), Turmeric rhizome extract (*C. longa*) (47.41%), Tulsi leaves extract (*O. sanctum*) (36.74%) and Nirgudi leaves extract (*Vitex* spp.) (32.30 %). The least inhibition of 15.07 % was seen with Shatavari leaf extract (*A. racemosus*). At 20 percent concentration, mycelial growth ranged from 5.00 mm (Garlic bulb extract) (*A. sativum*) to 61.63 mm (Shatavari leaves extract) (*A. racemosus*). However, significantly least mycelial growth was recorded which was 5.00 mm with Garlic bulb extract (*A. sativum*). This was followed by the botanicals *viz*., Neem leaves extract (*A. Indica*) (22.06 mm), Turmeric rhizome extract (*C. longa*) (23.30 mm), Ginger rhizome extract (*Z. officinale*) (29.33 mm), Onion bulb extract (*A. cepa*) (44.16 mm), Nirgudi leaves extract (*Vitex* spp.) (46.35 mm), Tulsi leaves extract (*O. Sanctum*) (52.56 mm. However, maximum mycelial growth was recorded which was 61.63 mm with Shatavari leaves extract (*A. racemosus* extract). Garlic bulb extract (*Allium sativum*) showed 94.44% significant inhibition of mycelium (*Colletotrichum orbiculare*) over all the treatments. The next effective phytoextracts at 20 percent concentration, in order of inhibition were Neem leaves extract (*A. indica*) (75.48 %), Turmeric rhizome extract (*C. longa)* (74.11%), Ginger rhizome extract (*Z. Officinalis*) (67.41%), Nirgudi leaves extract (*Vitex* spp.) (48.52%), Onion bulb extract (*A. cepa*) (50.93%), Tulsi leaves extract (*O. sanctum*) (41.60%) and Shatavari leaves extract (*A. racemosus*) (31.52%) was found least effective in inhibiting the mycelial growth of *Colletotrichum orbiculare*. These results supported the observations of reported Garlic extract at 20% concentration appeared to be best in inhibiting the radial growth and mycelial dry weight of *Colletotrichum dematium* followed by onion, ginger, and neem extracts.

Water extracts of seven different plants were tested against *C. lagenarium* (Gumme, 2011) [14] to check their anti-fungal properties Bulb extracts of *Allium sativum* recorded maximum inhibition (77.73%) followed by *Lawsonia inermis* (72.97%), *Azardirachta indica* (53.93), *Ocimum sanctum* (41.66%) and *Glyricidia sepium* (46.07).

Potphode (2011) evaluated six plant extracts at 10 % concentration against *Colletotrichum lagenarium* by using the poison food technique. Eucalyptus oil (10%) was found to be more effective in inhibiting mycelial growth (63.47%) *Colletotrichum lagenarium* followed by garlic (61.39%) and neem extract (60.56 %). Ginger showed the least (50.42%) inhibition of mycelia growth.

T_1:	*T. asperallum*	T_6:	*Aspergillus niger*
T_2:	*T. harzianaum*	T_7:	*Bacillus subtilis*
T_3:	*T. hamatum*	T_8:	*Pseudomonas fluorescens*
T_4:	*T. Koningii*	T_9:	Control
T_5:	*T. longibrachiatum*		

Fig 1: *In vitro* efficacy of bioagents on mycelial growth and inhibition of *Colletotrichum orbiculare*

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