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Potentiality of fungal and bacterial antagonists against *Fusarium pallidoroseum* (Cooke) Sacc. Causing wilt of coriander *in vitro*

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Abstract
Coriander (*Coriandrum sativum* L.) is an important annual herbaceous crop known to infect by various fungal plant pathogens. Among them, wilt caused by *Fusarium pallidoroseum* (Cooke) Sacc. marks the first reported instance affecting coriander in Saurashtra region. Looking to the importance of disease, attempts were made to evaluate potentiality of seven fungal and six bacterial antagonists against *F. pallidoroseum in vitro*. The results revealed that *Trichoderma koningii* among fungal antagonists exhibited maximum mycelial growth inhibition of 62.38 per cent *in vitro*. While, *Trichoderma hamatum* remained the least effective with 20.27 per cent. *Bacillus cereus* on the other hand showed maximum mycelial growth inhibition of 63.92 per cent among bacterial antagonists. Whereas, *Bacillus thuringiensis* remained the least effective with 27.94 per cent mycelial growth inhibition of *F. pallidoroseum* under laboratory condition.

Keywords: Coriander, *Fusarium pallidoroseum*, wilt, antagonists, mycelial growth inhibition

1. Introduction
Coriander (*Coriandrum sativum* L.) is a significant annual herbaceous plant native to the Mediterranean and Middle Eastern regions with a chromosome number of 2n=22. It is known by various names as Dhaniya in Hindi and Kotthamalli in Tamil. It is one of the oldest spices mentioned in history, with evidence of its use for more than 5000 years ago and referred to as "Spice of Happiness" by the Egyptians (Chahal *et al*., 2017) [5]. The crop is known to infect by various fungal diseases like, powdery mildew (*Erysiphe polygoni* DC.), wilt [*Fusarium solani* (Mart.) Sacc. or *F. oxysporum* f. sp. *coriandrii*] and root rot (*Rhizoctonia solani* Kuhn) (Lakra, 2001) [14]. Besides these, a novel reported wilt disease caused by *Fusarium pallidoroseum* (Cooke) Sacc. marks the first occurrence in Saurashtra region. Earlier, this fungus has been reported in West Bengal causing wilt disease of *Chlorophytum nepalense* (Bose *et al*., 2010) [4], fruit rot disease of citrus in Gujarat (Baria *et al*., 2015) [3] and wilt disease of chilli in Kashmir (Wani, 2007) [25]. Since it is a new pathogen identified in the Saurashtra (Pithiya and Kanzaria, 2024) [19], it is very necessary to develop an effective ecofriendly management strategy. Hence, the present study was framed in order to contribute new insights into disease management strategy against *Fusarium pallidoroseum* (Cooke) Sacc. causing wilt of coriander using fungal and bacterial antagonists *in vitro* by advocating the Materials and Methods given below.

2. Materials and Methods
A potentiality of seven fungal and six bacterial antagonists were evaluated against *F. pallidoroseum* in laboratory condition at Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh. The experiment was laid out in Completely Randomized Design with factorial concept.

2.1 Potentiality of fungal antagonists against *F. pallidoroseum in vitro*
The potentiality of seven fungal antagonists *viz., Trichoderma viride*, *T. harzianum*, *T. virens*, *T. koningii*, *T. hamatum*, *T. asperellum* and *T. viride* NAU isolate-1 (2×10^6 cfu/g

minimum) were tested against *F. pallidoroseum* using Dual Culture Technique of Morton and Stroube (1955) [16] with three repetitions. Twenty milliliters of sterilized melted potato dextrose agar (PDA) were poured aseptically in each 90 mm diameter Petri plate and allowed to solidify. Mycelial disc of four millimeters diameter of each antagonist and test fungus were cut with the help of sterilized cork-borer from the edges of actively growing culture were placed aseptically on the PDA medium in the same Petri plates, on opposite corners by keeping one cm distance from distal ends of Petri plates. A control plate was also maintained separately by placing two pathogens in the same plate, on the opposite corner. The inoculated plates were incubated at 28±1 °C in BOD incubator. The plates were observed periodically for the growth of the antagonist and test fungus.

2.2 Potentiality of bacterial antagonists against *F. pallidoroseum in vitro*

The potentiality of six bacterial antagonists *viz*., *Pseudomonas fluorescens* JAU isolate-1, *P. fluorescens* JAU isolate-2, *Bacillus cereus*, *B. subtilis* JAU isolate-1, *B. subtilis* JAU isolate-2 and *B. thuringiensis* were tested against *F. pallidoroseum* with four repetitions. The test fungus was dual culture plated with different six bacterial antagonist as described by Montealegre *et al.* (2003) [15]. Twenty milliliters of sterilized melted PDA (50%) + NA (50%) media were poured aseptically in each 90 mm diameter Petri plate and allowed to solidify. Mycelial disc of four millimeters diameter of test fungus was cut with the help of a sterilized cork-borer from the edges of actively growing culture and were placed aseptically in the center of PDA (50%) + NA (50%) medium containing Petri plates. A circular line made by dipping periphery of glass funnel of the size 60 mm diameter in a suspension of different bacterial antagonists (1×10^8 cfu/ml minimum) surrounding the fungal inoculum. The inoculated plates were then incubated for 72 h at 28±1 °C. A control plate was also maintained separately by placing pathogen in the centre of the Petri plate and the fungal growth was measured in all treatments and compared with control plate where the bacterial suspension replaced by sterile distilled water (Rupapara *et al*., 2019) [21].

The radial growth of the test pathogen was measured after five days of inoculation. The percent mycelial growth inhibition of the test fungus in each treatment by the fungal and bacterial antagonists were calculated by using the formula given here under (Vincent, 1947) [24].

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

Where,

I = Percent mycelial growth inhibition

C = Mean radial growth of test pathogen in control plate (mm)

T = Mean radial growth of test pathogen in treated plate (mm)

2.3 Statistical analysis

Statistical analysis was carried out as per standard methods as suggested by Panse and Sukhatme (1985) [17].

3. Results and Discussion

The observations on mycelial growth inhibition were recorded after five days of inoculation. The results obtained are communicated here under.

3.1 Potentiality of fungal antagonists against *F. pallidoroseum in vitro*

The data presented in Table 1, Figure 1 and Plate I indicated that all the antagonistic fungi were capable of reducing the mycelial growth of *F. pallidoroseum* over control. *Trichoderma koningii* gave maximum mycelial growth inhibition of 62.38 per cent and proved significantly superior over rest of the treatments. The next effective treatment in order of merit was *T. viride* NAU isolate-1 with 53.67 per cent mycelial growth inhibition, but it was remained statistically at par with *T. harzianum* with mycelial growth inhibition of 49.31 per cent. Whereas, *T. viride* found moderately effective treatment with mycelial growth inhibition of 36.25 per cent and was remained at par with *T. virens* (31.90%) followed by *T. asperellum* (28.99%). Whereas, *T. hamatum* found the least effective treatment with 20.27 per cent mycelial growth inhibition of *F. pallidoroseum*.

The present study was in line with the results obtained by Karpe (2021) [12]. He reported least growth of *F. oxysporum* f. sp. *coriandrii* in presence of *T. pseudokoningii in vitro.* The effectiveness of *T. koningii* also reported by Bammidi (2017) [2], Patra and Biswas (2017) [18] and Charan *et al.* (2021) [6]. Whereas, Ghaghara *et al.* (2019) [8] and Ram *et al.* (2022) [20] revealed minimum growth inhibition of *Fusarium* spp. using *Trichoderma hamatum* which support the present findings.

Antimicrobial activity of fungal antagonists seems to be due to competition for food, production of antimicrobial compounds and mycoparasitism as suggested by (Sivan and Chet, 1989) [22] while employing *T. harzianum* for the control of *F. oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis* in the cotton and melon rhizosphere, respectively.

The role of secondary metabolites such as alkyl pyrones, iso-nitriles, polyketides, petaibols, diketopiperazines, sesquiterpenes and steroids in inhibition of microbial activities were also reported by (Howell, 1998) [10]. Sonkar *et al*., 2018 [23] showed trichodermin, viridin, harzinic acid, siderophore and gliotoxin as the toxic compound against *Fusarium oxysporum*. Similarly, Haran *et al*. (1996) [9] proposed the mycoparasitic activity of *Trichoderma* as one of the major mechanisms involved in their antagonistic activities against phytopathogenic fungi.

3.2 Potentiality of bacterial antagonists against *F. pallidoroseum in vitro*

The data presented in Table 2, Figure 2 and Plate II indicated that all the bacterial antagonists significantly inhibited the mycelial growth of *F. pallidoroseum* over control. The maximum mycelial growth inhibition of 63.92 per cent was observed in *Bacillus cereus* and was proved significantly superior over rest of treatments. The next effective treatment in order of merit was *Pseudomonas fluorescens* JAU isolate-1 with 61.11 per cent mycelial growth inhibition followed by *Pseudomonas fluorescens* JAU isolate-2 (55.22%). The treatment *Bacillus subtilis* JAU isolate-1 found moderately effective with mycelial growth inhibition of 40.06 per cent followed by *Bacillus subtilis* JAU isolate-2 (32.38%). Whereas, *Bacillus thuringiensis* found the least effective treatment with 27.94 per cent mycelial growth inhibition of *F. pallidoroseum*.

A more or less similar kinds of results were also reported by Charan *et al.* (2021) [6]. He reported the effectiveness *Bacillus cereus* in reducing mycelial growth of *F. oxysporum* f. sp. *cumini in vitro*. The effectiveness of *Pseudomonas fluorescens* and *Bacillus subtilis* also reported by Baria *et al.* (2015) [3], Kumar *et al.* (2016) [13] and Jangir (2021) [11], which support the results of present findings.

The bacterial antagonists *Pseudomonas* spp. produces phenazines, phloroglucinols, dialkyl-resorcinols, pyoluteorin and pyrrolnitrin like compounds which act as mechanism of action in biological control (Chin-A-Woeng *et al*., 2003) [7]. *Bacillus* spp. produce several bacteriocins with antimicrobial activity such as amylolysin, amylocyclicin, amysin, subtilin, subtilosin A, subtilosin B and thuricin (Abriouel *et al*., 2011) [1].

Table 1: Potentiality of fungal antagonists against *F. pallidoroseum*

Tr. No.	Fungal antagonists	Mycelial growth inhibition (%)
1.	*Trichoderma viride*	37.02 (36.25)
2.	*Trichoderma harzianum*	44.61 (49.31)
3.	*Trichoderma virens*	34.39 (31.90)
4.	*Trichoderma koningii*	52.16 (62.38)
5.	*Trichoderma hamatum*	26.76 (20.27)
6.	*Trichoderma asperellum*	32.58 (28.99)
7.	*Trichoderma viride* NAU isolate-1	47.10 (53.67)
	S. Em. ±	0.90
	C. D. at 5%	2.71
	C. V.%	3.95

Note: Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

Table 2: Potentiality of bacterial antagonists against *F. pallidoroseum*

Tr. No.	Bacterial antagonists	Mycelial growth inhibition (%)
1.	*Pseudomonas fluorescens* JAU isolate-1	59.46 (61.11)
2.	*Pseudomonas fluorescens* JAU isolate-2	53.56 (55.22)
3.	*Bacillus cereus*	62.27 (63.92)
4.	*Bacillus subtilis* JAU isolate-1	38.41 (40.06)
5.	*Bacillus subtilis* JAU isolate-2	30.72 (32.38)
6.	*Bacillus thuringiensis*	26.29 (27.94)
	S. Em. ±	0.56
	C. D. at 5%	1.65
	C. V.%	2.47

Note: Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values

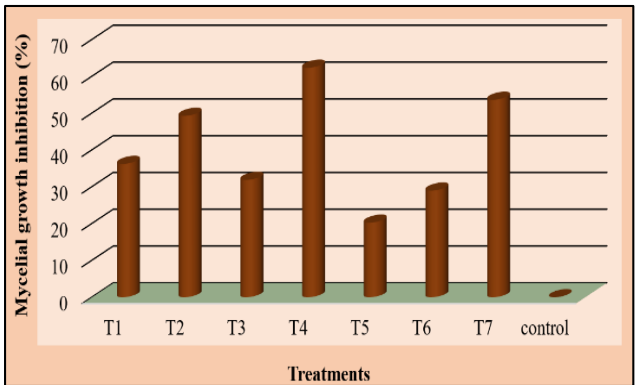

Fig 1: Potentiality of fungal antagonists against *F. pallidoroseum*

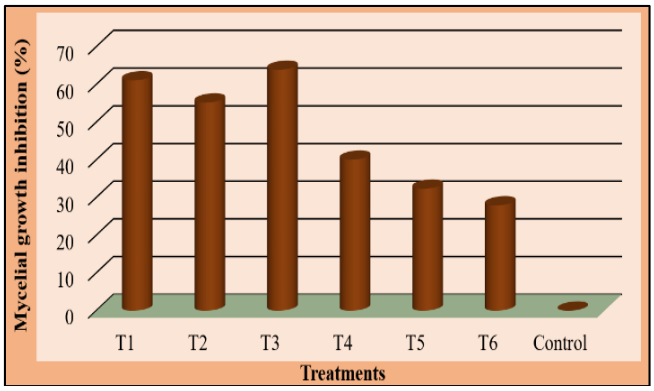

Fig 2: Potentiality of bacterial antagonists against *F. pallidoroseum*

Fungal antagonists
T1: *Trichoderma viride*
T2: *Trichoderma harzianum*
T3: *Trichoderma virens*
T4: *Trichoderma koningii*
T5: *Trichoderma hamatum*
T6: *Trichoderma asperellum*
T7: *Trichoderma viride* NAU isolate-1
T8: Control

Plate I: Potentiality of fungal antagonists against *F. pallidoroseum*

Bacterial antagonists
T1: *Pseudomonas fluorescens* JAU isolate-1
T2: *Pseudomonas fluorescens* JAU isolate-2
T3: *Bacillus cereus*
T4: *Bacillus subtilis* JAU isolate-1
T5: *Bacillus subtilis* JAU isolate-2
T6: *Bacillus thuringiensis*
T7: Control

Plate II: Potentiality of bacterial antagonist against *F. pallidoroseum*

4. Conclusion

The present study revealed that all the fungal and bacterial antagonists were capable of reducing the mycelial growth of *Fusarium pallidoroseum* to different degrees. Among the fungal antagonists tested *in vitro*, *Trichoderma koningii* and *Bacillus cereus* among bacterial antagonists found the most effective antagonists in inhibiting the mycelial growth of *F. pallidoroseum* under laboratory condition.

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