



Effect of Selected Bio-agents and Botanical on Alternarial Leafspot of Coriander (*Coriandrum sativum* L.) Caused by *Alternaria alternata* (Fries) Keissler

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Coriander (*Coriandrum sativum* L.) is one of the important spice crops of Apiaceae family. Coriander seeds are used as medicine to cure indigestion, dysentery, vomiting as well as cold and like all other green leafy vegetables, its leaves are a rich source of vitamins, minerals and iron. The country's annual production of coriander seeds in the year 2021 was over 822 thousand metric tons cultivated over 2.92 lakh hectares. Alternarial leaf spot of Coriander which is caused by *Alternaria alternata* is one major disease in the coriander. The *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis* and Garlic extract were tested under field conditions during rabi season 2021 for their efficacy against the disease and growth & yield parameters. Among the treatments the maximum plant height (cm) at 90 DAS was recorded in T₂ – *Trichoderma viride*+ *Pseudomonas fluorescens* followed by T₃ – *Pseudomonas fluorescens* as compared to untreated check control T₀. The maximum number of branches at 45 DAS was recorded in T₂ – *Trichoderma viride*+ *Pseudomonas fluorescens* followed by T₃ – *Pseudomonas fluorescens* as compared to untreated check control T₀. The maximum root length at 90 DAS was recorded in T₅– *Trichoderma viride*+ *Bacillus subtilis* followed by T₂ – *Trichoderma viride* + *Pseudomonas fluorescens* as compared to

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untreated check control T₀. The minimum disease intensity (%) at 75 DAS was recorded in T₂ – *Trichoderma viride*+ *Pseudomonas fluorescens*, followed by T₁–*Trichoderma viride* as compared to untreated check control T₀. The maximum yield (q/acre) of onion was recorded T₄ –*Trichoderma viride* +*Pseudomonas fluorescens* followed by T₁ – *Trichoderma viride* as compared to untreated control T₀.

Keywords: *Trichoderma viride*; *Pseudomonas fluorescens*; garlic extract oil; *Alternaria alternata*; *Bacillus subtilis*.

1. INTRODUCTION

Coriander is one of the important spice plants [1] and referred to as "kusthumbari" or "dhanayaka" in the Sanskrit literature; in Hindi it is called Dhania, while Dhane in Bengali. It is a native plant of the eastern Mediterranean from where it may have spread to India and the rest of the world. Coriander (*Coriandrum sativum* L.), an annual of the Apiaceae family, is one of the important spice plants [1]. "India leads in the production of coriander and covers 53.3% of the market share of the world and there has been a growth of 15.8% in its production. In India crop is grown in almost all the states of the country but Rajasthan, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Orissa, Uttar Pradesh, and Uttarakhand are the major coriander-growing states. Madhya Pradesh produced the largest volume of coriander seeds in the fiscal year 2020 across India. This amounted to over 370 thousand metric tons, over an area of 628 thousand hectares. The country's annual production of coriander seeds that year was over 755 thousand metric tons. Uttar Pradesh produced 5.26 thousand metric tons. This amounted to over 370 thousand metric tons, over an area of 628 thousand hectares. The country's annual production of coriander seeds was over 755 thousand metric tons. For leaf purposes, coriander is grown all year round. It can fairly tolerate light frost and high temperatures. Heavy rain affects crop yield and quality badly. In very hot weather, the crop for leaves can be grown under assured irrigation facilities" [2]. "Coriander contains 24 g carbohydrates, 1.3 g protein, 19.6 g fat, 5.3 g minerals and 6.3 g moisture in 100 g seed. Other compounds are linalool, a and b pinene, para cymene, a- terpinene. The oil from foliage contains aliphatic aldehydes, mainly dacylaldehyde. The seeds have 0.4 per cent essential oil. Linalool is the main component of up to 90% and up to 7% thymol. Oleresins with 90% fatty oil and 5% steam volatile oil is also obtained from seeds. Coriander seeds are used as medicine to cure indigestion, dysentery, vomiting as well as cold. The essential oil has

carminative, antiseptic, bactericidal, fungicidal , and muscle relaxant" [2].

"The coriander plant is mainly used for making sauces and salads on the other hand, the fruits are blended into powder for flavoring various products like meat, fish, sodas, pickles, bakery and curry recipes" [3]. "The coriander plant parts and seeds are used by people as short-cut medicines for various body problems" [2]. "Like all other green leafy vegetables, its leaves are a rich source of vitamins, minerals, and iron. Its leaves contain a high amount of vitamin A (β -carotene) and vitamin C. The green herbs contain vitamin C up to 160 mg/100 g and vitamin A up to 12 mg/100 g. Coriander oil contains coriandrol, girenol and vebriniol" [4]. Chemicals derived from coriander leaves were found to have antibacterial activity against *Salmonella choleraesuis* sp. *choleraesuis* .

"Despite the economic importance of coriander, current production trends are below the crops' genetic potential due to several biotic and abiotic stresses. Among biotic factors are some diseases caused by various fungi. [5]. reported a 60% loss of coriander yield by a wilting disease caused by *Fusarium oxysporum* f. sp. *coriandrii* (Fusacr). Although plants infected with *Alternaria spp*". "The pathogen seems to have the adaptability to higher temperatures and the disease occurs during February-April, and it is particularly severe at flowering and post-flowering stages causing considerable losses to the yield (25-40%) and also producing very low quality. Although plants infected with *Alternaria* sp. seldom die, the presence of lesions and other foliar blemishes may significantly reduce their market value" [6,7,8]. In coriander, common symptoms of alternarial leaf spot are Small dark-colored circular spots produced on leaves and green stems. Concentric rings are also observed at certain times. The disease harms yield. High moisture favors the disease.

"Morphology of the pathogen of the genus *Alternaria* was first described by Nees in 1817

with *Alternaria tenuis* as the type species [9]. The conidial characteristics of the genus are uniform, attenuated and catenulate. (Fries, 1832). had proposed the *Alternaria alternata* as *Torula alternata* Pers. Under *in vitro* conditions, sporulation occurs at a temperature range 8-24°C, where mature spores sporulate after 14 to 24 hours. The optimum temperature is between 16 and 24°C where sporulation time ranges from 12 to 14 hours and moisture in the presence of rain, dew or high humidity is essential for infection and a minimum of 9-18 hours is required for the majority of the species". Continuous moisture of 24 hours or longer practically guarantees infection.

"This present study seeks to determine the effect of non-chemical seed treatments, viz. biocontrol agents *Trichoderma viride*, *Bacillus subtilis*, and *Pseudomonas fluorescens*. Plant extracts (garlic extract) were studied as alternatives to synthetic chemicals for the management of *Alternaria alternata*" (Fr.) Keissl. Causing, Alternarial leaf spot affecting coriander, the main aim of this study is to test and compare the inhibitory effect of bio control agents and botanical against *Alternaria alternata* and evaluate their potential application to manage leaf spot of coriander.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted at the laboratory of the Department of Plant Pathology and field experiment was carried out at the Central Research Field, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj during Rabi season 2020-2021.

2.2 Methodology

2.2.1 Collection of disease samples

Plants showing typical symptoms, in the field of standing crop, that is the infected plant part of Coriander was selected. These disease plant materials were brought to the lab for further investigation.

2.2.2 Identification of the fungus by slide preparation

"Examination of the fungal colony characteristics was done through microscopic examination. Using a sterile needle, a small portion of the

infected plant part was taken and placed on a sterile glass slide. It was stained using lactophenol and cotton blue and covered with the coverslip. Then, the microscope was used for the examination of morphological characteristics of fungal structures" (Rao et al. 2021).

Morphological Characters of *Alternaria alternata*:

The conidia are single or in chains of 2-8 spores, they are smooth with a rounded base and tapering towards the apex, which may have a septate or non-septate beak. They possess 2-10 transverse and 1-3 longitudinal septa. The conidiophores arise in single or in a cluster, usually, 2-6 is branched, erect, straight, or irregularly bent [10].

2.2.3 Evaluation of bioagents and botanical against *Alternaria alternata* In vivo

The efficacy of bioagents and botanical against *Alternaria alternata* was carried out in field conditions.

2.2.4 Observations recorded

Pre-harvest observations were recorded during the course of experiment. Pre-harvest observations were Mid-stem length, number of branches per plant, root length per plant, Disease intensity.

2.2.5 Disease intensity

Disease intensity (%) formula was given by Wheeler (1969) disease intensity was used for calculation:

$$\text{Disease intensity (\%)} = \frac{\text{sum of all disease ratings}}{\text{Total number of Rating} \times \text{Maximum disease grade}} \times 100$$

3. RESULTS

3.1 Mid Stem Length (cm)

The mid stem length significantly increased in combined treatment T2 *Trichoderma viride* + *Pseudomonas fluorescens* (83.56cm) followed by T4 Garlic extract (77.86cm), T3 *Pseudomonas fluorescens* (77.667cm), T5 *Trichoderma viride* + *Bacillus subtilis* (76.1cm) when compared to T1 *Trichoderma viride* (72.10), and in T0 untreated control (63.00cm) Among the treatments (T1,T5), (T5, T3) and (T3, T4) were found non-significant to each other.

Table 1. Effect of bio agents and botanical on mid stem length (cm), number of branches per plant and root length per plant

Tr. no	Treatment	Mid stem length (cm)			No of Branches (no)	Root length (cm)
		30 DAS	60 DAS	90 DAS	45 DAS	
T ₀	Control	11.333	24.000	63.000	4.000	3.067
T ₁	<i>Trichoderma viride</i>	15.033	33.100	72.100	8.000	5.233
T ₂	<i>Trichoderma viride</i> + <i>Pseudomonas fluorescens</i>	22.233	44.567	83.567	11.000	5.900
T ₃	<i>Pseudomonas fluorescens</i>	20.733	38.667	77.667	10.000	5.633
T ₄	Garlic extract	14.200	38.867	77.867	6.333	4.400
T ₅	<i>Trichoderma viride</i> + <i>Bacillus subtilis</i>	17.767	37.133	76.133	8.333	6.567
F Test		S	S	S	S	S
C.D.		2.295	5.402	5.603	2.888	1.318
S.Ed.(±)		1.017	2.394	2.494	1.279	0.584

3.2 No. of Branches

The maximum plant branches significantly increased in combined treatment T₂ *Trichoderma viride* + *Pseudomonas fluorescens* (11.00) Followed by T₃ *Pseudomonas fluorescens* (10.0), T₅ *Trichoderma viride* + *Bacillus subtilis* (8.33) treatments, T₁ *Trichoderma viride* (8.0) when compared to T₄ Garlic extract (6.33) and in T₀ untreated control (3.00). Among the treatments (T₄,T₁), (T₁, T₅), (T₅, T₃) and(T₃, T₂) are non-significant to each other.

3.3 Root Length

The root length of Coriander significantly maximum in combined treatments T₅ *Trichoderma viride* + *Bacillus subtilis* (6.56 cm) followed by T₂ *Trichoderma viride* + *Pseudomonas fluorescens* (5.900 cm), T₃ *Pseudomonas fluorescens* (39.733cm), T₁ *Trichoderma viride* (38.133cm) ,when compared to treatments T₄ Garlic extract (4.400 cm) , in T₀ untreated control (3.06cm). Among the treatments (T₄, T₁) (T₁, T₃) (T₃, T₂) (T₂, T₅) are non-significant to each other.

Table 2. Effect of treatments on Alternarial leaf spot disease intensity at 45 ,60 and 75 DAS of Coriander (*Coriandrum sativum* L.)

Treatment no	Treatment	Disease intensity (%)		
		45 DAS	60 DAS	75 DAS
T ₀	Control	32.000	38.400	52.800
T ₁	<i>Trichoderma viride</i>	17.733	22.133	38.133
T ₂	<i>Trichoderma viride</i> + <i>Pseudomonas fluorescens</i>	15.733	17.867	27.933
T ₃	<i>Pseudomonas fluorescens</i>	18.433	20.000	39.733
T ₄	Garlic extract	21.067	26.933	43.200
T ₅	<i>Trichoderma viride</i> + <i>Bacillus subtilis</i>	16.433	24.800	40.267
Ftest		S	S	S
C.D.		2.529	3.041	1.490
S.Ed. (±)		1.121	1.347	0.660

3.4 Disease Intensity

The disease intensity (%) significantly decreased in combined treatment was T2 *Trichoderma viride* + *Pseudomonas fluorescens* (27.933 followed by) T1 *Trichoderma viride* (38.133), T3 *Pseudomonas fluorescens* (39.733), T5 *Trichoderma viride* + *Bacillus subtilis* (40.267), compared to treatments T4 Garlic extract (43.200) and in T0 untreated control (52.800). Among the treatments (T3, T5) is non-significant to each other but significant to other.

4. DISCUSSION

“*Trichoderma* species produce secondary metabolites such as antibiotics, isocyanide, acids and cell wall degrading enzymes which are implicated in the growth inhibition of many phytopathogenic fungi” [11]. “*Pseudomonas fluorescens* have been shown potential agents for the biocontrol which suppress plant diseases by protecting the seeds, and roots from fungal infection” [12]. Garlic extract may give good inhibition to spore germination due to presence of allicin in garlic responsible for bursting the young hyphae of fungus. And present findings are similar to the work reported by Bochalya et al., 2012 *Trichoderma* fungi, “it possesses many qualities and they have great potential use in agriculture such as amending abiotic stresses, improving physiological response to stresses, alleviating uptake of nutrients in plants, enhancing nitrogen-use efficiency. *Pseudomonas* promotes plant growth by suppressing pathogenic micro-organisms, synthesizing growth-stimulating plant hormones, and promoting increased plant disease resistance [13-16]. *Pseudomonas fluorescens* have been shown to be potential agents for bio-control which suppresses plant diseases by protecting the seeds and roots from fungal infection. *Bacillus* species that can form spores and survive in the soil for a long period under harsh environmental conditions [17-19]. Plant growth is enhanced by PGPR through the induction of systemic resistance, antibiosis, and competitive omission”. *Bacillus* species have become attractive biological control agents due to their ability to produce hard, resistant endospores and antibiotics which control a broad range of plant pathogens. And the present findings are the same as [3].

5. CONCLUSION

The *In vitro* study reveals that, on basis of observations, it is shown that among all

treatments T2 – *Trichoderma viride* + *Pseudomonas fluorescens* @ 5% gave the best results in comparison to other treatments, where it has given highest length of mid stem height (83.56) cm, The maximum number of branches (11.0), The minimum disease intensity (30.93) when compared with other treatments. The maximum root length recorded in the treatment T5– *Trichoderma viride* + *Bacillus subtilis* @ 5% (6.5) The lowest mid-stem height, minimum no of branches, and maximum disease intensity was recorded in T0 control followed by T₄ garlic extract at @5%.

Hence, from the present study it can be concluded that T2 – *Trichoderma viride* + *Pseudomonas fluorescens* @ 5% and T2 *Trichoderma viride*, T5 *Trichoderma viride* + *Bacillus subtilis* are the best alternative treatment for chemicals to reduce the disease intensity of alternarial leaf spot disease and to get better yield.

As it is beneficial and ecofriendly, easy to get for farmers therefore, it may be recommended for the better management of Alternarial leaf spot of coriander. Results of the present study found to be significantly effective under prayagraj Agroclimatic conditions. it may vary with region and climatic conditions, therefore for the validations of the results more such trails should be carried out in future for further recommendation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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