

## Effect of selected bio-agents and botanical on Alternarial leafspot of coriander (*Coriandrum sativum* L.)

### Abstract :

Coriander (*Coriandrum sativum* L.) is one of the important spice crops belong to Apiaceae family. Coriander seeds are used as medicine to cure indigestion, dysentery, vomiting as well as cold and flu. Like all other green leafy vegetables, its leaves are a rich source of vitamins, minerals and iron. Its leaves contain high amounts of vitamin A ( $\beta$ -carotene) and vitamin C. The country's annual production of coriander seeds in the year 2021 was over 822 thousand metric tons cultivated over 2.92 lakh hectares. Current production trends are below the crops genetic potential due to Pest and diseases caused by various fungi. 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 T<sub>2</sub> – *Trichoderma viride*+ *Pseudomonas fluorescens* followed by T<sub>3</sub> – *Pseudomonas fluorescens* as compared to untreated check control T<sub>0</sub>. The maximum number of branches at 45 DAS was recorded in T<sub>2</sub> – *Trichoderma viride*+ *Pseudomonas fluorescens* followed by T<sub>3</sub> – *Pseudomonas fluorescens* as compared to untreated check control T<sub>0</sub>. The maximum root length at 90 DAS was recorded in T<sub>5</sub>– *Trichoderma viride*+ *Bacillus subtilis* followed by T<sub>2</sub> – *Trichoderma viride*+ *Pseudomonas fluorescens* as compared to untreated check control T<sub>0</sub>. The minimum disease intensity (%) at 75 DAS was recorded in T<sub>2</sub> – *Trichoderma viride*+ *Pseudomonas fluorescens*, followed by T<sub>1</sub>–*Trichoderma viride* as compared to untreated check control T<sub>0</sub>. The maximum yield (q/acre) of onion was recorded T<sub>4</sub> –*Trichoderma viride* +*Pseudomonas fluorescens* followed by T<sub>1</sub> – *Trichoderma viride* as compared to untreated control T<sub>0</sub>.

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

### INTRODUCTION

Comment [L1]: Italic

Comment [L2]: Which country?

Comment [L3]: All of these are not needed in Abstract.

Comment [L4]: Italics

Comment [L5]: and

Coriander is one of the important spice plants. (Coskuner and Karababa, 2007) 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, China, and the rest of the world. Coriander (*Coriandrum sativum* L.), an annual of the Apiaceae family, is one of the important spice plants. (Coskuner and Karababa, 2007). Coriander is distributed worldwide. 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. Coriander, according to the climatic conditions, is cultivated as a summer or winter annual, tropical or subtropical crop and can tolerate heat and drought. For leaf purposes, coriander is grown all year round. It can fairly tolerate light frost and high temperatures. The temperature of 15-25°C for vegetative growth and 20-30°C along with cool and dry weather for seed formation is considered good. Heavy rain affects crop yield and quality badly. In very hot weather, the crop for leaves can be grown under assured irrigation facilities (Singh and Bhandari, 2015). 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. (Singh *et al.*, 2007).

The coriander plant is mainly used for making sauces and salsas; on the other hand, the fruits are blended into powder for flavoring various products like meat, fish, sodas, pickles, bakery and

Comment [L6]: italics

Comment [L7]: do you mean a member of Apiaceae family .....

Comment [L8]: unbold

Comment [L9]: unbold

curry recipes (**Ravi et al., 2007**). The coriander plant parts and seeds are used by people as short-cut medicines for various body problems (**Singh et al., 2007**). 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 ( $\beta$ -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 is an important source of chemicals like alpha-pinene, gamma terpinene, linalool, cymene, various non-linalool alcohols, esters, flavonoids, coumarins, isocoumarins, phthalides and phenolic acids. Coriander oil contains coriandrol, gireniol and vebriniol. (**Pandey, 2010**). 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. (**Manoranjitham et al., 2003**). 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 (**Hashmi and Ghaffar, 1991; Boedoet al., 2012 and Mangwende et al., 2018**). 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 (**Nees, 1817**). 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

Comment [L10]:

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 efficacy of In this regard, non-chemical seed treatments, viz. biocontrol agents *Trichoderma viride*, *Bacillus subtilis*, and *Pseudomonas fluorescens*. plant extracts were studied as alternatives to synthetic chemicals for the management of *Alternaria alternata* (Fr.) Keissl. Causing, Alternarial leaf spot affecting coriander.

Comment [L11]: please rewrite

Therefore the main aim of this study is to test and compare the inhibitory effect of Three bio control agents and botanical (garlic extract against *Alternaria alternata* and evaluate their potential application to manage leaf spot of coriander.

Comment [L12]: merge paragraph

## Materials and Methods

### 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.

### Methodology:

#### Collection of disease samples:

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

#### 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.

**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 (Chowdhary and Varshney, 2000)

**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.

**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.

**Disease intensity**

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

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

**Results**

**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	Root length
		30 DAS	60 DAS	90 DAS		
T0	Control	11.333	24.000	63.000	4.000	3.067
T1	<i>Trichoderma viride</i>	15.033	33.100	72.100	8.000	5.233
T2	<i>Trichoderma viride</i> + <i>Pseudomonas fluorescens</i>	22.233	44.567	83.567	11.000	5.900
T3	<i>Pseudomonas fluorescens</i>	20.733	38.667	77.667	10.000	5.633
T4	Garlic extract	14.200	38.867	77.867	6.333	4.400
T5	<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

**Comment [L13]:** If you look at this table, a number of corrections has been suggested to: Makes the table looks scientific using only 3 lines separation Secondly, reduce it space while the content remains well arranged and visible.  
Please let other tales follow the same format.

**Mid stem length (cm)**

The mid stem length of Coriander significantly increased in combined treatment T2 *Trichoderma viride*+ *Pseudomonas fluorescens*(83.56) followed by T4 Garlic extract (77.867), T3 *Pseudomonas fluoscens*(77.667), T5 *Trichoderma viride*+ *Bacillus subtilis* (76.1) when compared to T1 *Trichoderma viride*(72.10), and in T0 untreated control (63.00)Among the treatments (T1,T5), (T5, T3) and (T3, T4) were found non-significant to eachother.

**No. of branches**

The maximum plant branches of Coriander significantly increased in combined treatment T2 *Trichoderma viride*+ *Pseudomonas fluorescens*(11.00)Followed by T3 *Pseudomonas fluoscens*(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.

**Root length**

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

**Comment [L14]:** All of these could be reported in one paragraph

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

	Treatment	Disease intensity
--	-----------	-------------------

Treatment no		45 DAS	60 DAS	75 DAS
1	Control	32.000	38.400	52.800
2	<i>Trichoderma viride</i>	17.733	22.133	38.133
3	<i>Trichoderma viride</i> + <i>Pseudomonas fluorescens</i>	15.733	17.867	27.933
4	<i>Pseudomonas fluorescens</i>	18.433	20.000	39.733
5	Garlic extract	21.067	26.933	43.200
6	<i>Trichoderma viride</i> + <i>Bacillus subtilis</i>	16.433	24.800	40.267
	<b>F test</b>	<b>S</b>	<b>S</b>	<b>S</b>
	<b>C</b>	2.529	3.041	1.490
	<b>D</b>			
	<b>S</b>	1.121	1.347	0.660
	<b>E</b>			
	<b>d</b>			
	<b>(</b>			
	<b>±</b>			
	<b>)</b>			

Comment [L15]: Use the format in table 1

### Disease Intensity

The disease intensity(%)significantly decreased in combined treatment was T<sub>2</sub> *Trichoderma viride*+ *Pseudomonas fluorescens*(27.933followed by) T<sub>1</sub> *Trichoderma*

*viride*(38.133) , T<sub>3</sub> *Pseudomonas fluorescens*(39.733), T<sub>5</sub> *Trichoderma viride*+ *Bacillus subtilis* (40.267), compared to treatments T<sub>4</sub> Garlic extract (43.200) and in T<sub>0</sub> untreated control (52.800). Among the treatments (T<sub>3</sub>, T<sub>5</sub>) is non-significant to each other but significant to other.

### **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 (**Hariprasad et al., 2021**) *Pseudomonas fluorescens* have been shown potential agents for the biocontrol which suppress plant diseases by protecting the seeds, and roots from fungal infection (**Kakraliyet et al., 2017**) Garlic extract may give good inhibition to spore germination due to presence of allicin in garlic and protocatechuic acid, which were 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 in different crops, and assisting to improve photosynthetic efficiency. *Pseudomonas* promotes plant growth by suppressing pathogenic micro-organisms, synthesizing growth-stimulating plant hormones, and promoting increased plant disease resistance. *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. They are known to enhance plant growth promotion and reduce the severity of many fungal diseases. *Bacillus species* are a major type of rhizobacteria that can form spores and survive in the soil for a long period under harsh environmental conditions. Plant growth is enhanced by PGPR through the induction of systemic resistance, antibiosis, and competitive omission. *Bacillus subtilis* exhibits both direct and indirect biocontrol mechanisms to suppress disease caused by pathogens. *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 (**Ravishankar and Shashi Tiwari, 2018**)

## Conclusion:

The *In vitro* study reveals that, on basis of observations, it is shown that among all treatments T<sub>2</sub> – *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). and The minimum disease intensity (30.93) when compared with other treatments. The maximum root length recorded in the treatment T<sub>5</sub> – *Trichoderma viride* + *Bacillus subtilis* @ 5% (6.5) The lowest mid-stem height, minimum no of branches, and maximum disease intensity was recorded in T<sub>0</sub> control followed by T<sub>4</sub> garlic extract at @5%.

Hence, from the present study it can be concluded that T<sub>2</sub> – *Trichoderma viride* + *Pseudomonas fluorescens* @ 5% and T<sub>2</sub> *Trichoderma viride*, T<sub>5</sub> *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.

Comment [L16]: Please merge paragraph

## References:

Altinok, H. H. and Erdogan, O. (2015) Determination of the in vitro effect of *Trichoderma harzianum* on phytopathogenic strains of *Fusarium oxysporum*. *Notulae Botanicae Horti Agrobotanici Cluj Napoca* 43(2):494–500

Abdalla, S. A., Algam, S. A. A., Ibrahim, E. A. and Naim, A. M. E. (2014). *In vitro* screening of *Bacillus* isolates for biological control of early blight disease of tomato in Shambat soil. *World Journal of Agriculture Research* 2(1):47–50.

Abiodun, J., Osaretin, B. I., Elizabeth, T. A., Benson O. A. and Ajibola P. A. (2017). Effectiveness of *Pseudomonas species* in the management of tomato

early blight pathogen *Alternaria solani*. *African Journal of Microbiology Research*. 11(23) : 972-976

**Adarsh Pandey (2010)** Antagonism of two *Trichoderma* Species against *Alternaria alternata*

*Capsicum frutescens*. *Journal of Experimental Sciences* 1: 18-19 .

**Boedo, C., Benichou, S., Berruyer, R., Bersihand, S., Dongo, A., Simoneau, P. and Poupard,**

**P. (2012)**. Evaluating aggressiveness and host range of *Alternaria dauci* in a controlled environment. *Journal of Plant Pathology*. 61: 63-75

**Coskuner, Y. and Karabaaba, E. (2007)**. Physical properties of coriander seeds (*Coriandrum sativum*). *Journal of Food Engineering* . 80:408-16

**Chowdhry, P. N. and Varshney, A. (2000)**. Identification of Different *Colletotrichum gloeosporioides* species. in manual on identification of Plant pathogenic and biocotrol fungi of Agricultural importance. pp 73-78

**Hari Prasad, K., Palakshappa, M. G., Dinesh and Krishnananda, K. (2021)** SI Efficacy of bio-control agents under in vitro against *Alternaria porri* (Ellis). causing purple blotch in onion. *The Pharma Innovation Journal* 10(4):81-84.

**Hashmi, H. M. and Ghaffar, A. (1991)**. *Phoma multirostrata* on coriander in Pakistan. *Pakistan Journal of Botany*. 23: 127-130.

**Kakraliya, S. S., Choskit, D., Devanshi, P. and Sonali, A. (2017)**. Effect of bio-agents, neem leaf extract and fungicides against *Alternaria* leaf blight of wheat (*Triticum aestivum* L.). *Journal of Plant Pathology*. 5(8):100-106.

**Mangwende, E., Kritzing, Q., Truter, M. and Aveling, T. A. S.**

**(2018)**. *Alternaria alternata*: A new seed-transmitted disease of coriander in South Africa. *European Journal of Plant Pathology*. 152:409-416.

**Manoranjitham, K. S., Rabindran, R. and Doraiswamy, S. (2003).** Management of seed-borne pathogens and wilt disease of coriander. *The Madras Agricultural Journal*.90: 298–300.

**Mangwende, E., Kritzing, Q., Truter, M. and Aveling, T. A. S. (2018).** *Alternaria alternata*: A new seed-transmitted disease of coriander in South Africa. *European Journal of Plant Pathology*. 152:409-416.

**Manoranjitham, K. S., Rabindran, R. and Doraiswamy, S. (2003).** Management of seed-borne pathogens and wilt disease of coriander. *The Madras Agricultural Journal*.90: 298–300.

**Nees, C. G. (1817).** Quoted by Neergard. *Das System der Pilze und Schwamme*. 72.

**Ravi, R., Prakash, M. and Bhat, K. K. (2007).** Aroma characterization of coriander (*Coriandrum Sativum*L) oil samples. *European Journal of Food Research and Technology*.225(3-4): 367-374.

**Ravishankar, L. V. and Shashi Tiwari (2018).** Biological management of *Alternaria* leaf blight in coriander (*Coriandrum sativum*). *Journal of Pharmacognosy and Phytochemistry*.7(16):1867-1869

**Singh, K. P. and Bhandari, R. R. (2015).** Vegetable crops production technology. Samikshya publication, Khathmandu 103-107.

**Saravanakumar, K., Yu, C., Dou, K., Wang, M., Li, Y. and Chen, J.(2016) .** Synergistic effect of *Trichoderma*-derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of *Fusarium oxysporum f. sp. cucumerinum*.37-46

**Comment [L17]:** Aline spacing and reduce unnecessary gaps