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Biological management of Alternaria leaf blight in coriander (*Coriandrum sativum*)

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Abstract
Coriander is the medicinal and culinary plant and belongs to Umbelliferaceae family and it is well grown in Mediterranean type of climate and the seed have high demend in culinary and medicinal uses, the coriander suffers from many important disease like Alternaria blight, *Protomyces macrosporus*, and Powdery mildew. the present research was conducted to control the Coriander Alternaria blight caused by *Alternaria alternata* which is very important disease in reducing the quality of the seed, the present research done on central research field using bio agents and combitions in *in-vivo* and *in-vitro* conditions, *Trichoderma viride, Pseudomonas fluorescens* and Neem oil and their combinations the results shows the combination of bioagents had a great impact in Controling the blight disease of Alternaria blight followed by fungicide taken as check.

Keywords: *Alternaria alternata, Pseudomonas fluorescens, Trichoderma viride*

1. Introduction
Coriander (*Coriandrum sativum* L.), a medicinal and culinary plant from the Umbelliferaceae family, it is an annual herb belongs to the carrot family. Its name is derived from the Greek word "Koris" because of the unpleasant and bug like odour of the green herb from un-ripened fruits, India accounts for approximately 80 percent of the total world Coriander production.
Coriander has been used as a folk medicine for the relief of anxiety and insomnia. It is also used as a carminative, diuretic, stomach-ache and digestive aid. Coriander has been documented as a traditional treatment for diabetes, as eye wash for preservation of eyesight in smallpox and also in conjunctivitis. Chemicals derived from coriander leaves were found to have antibacterial activity against *Salmonella choleraesuis sp. choleraesuis* (Saskatchewan Herb & Spice Association, 2007).
The pathogen seems to have 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 produces a very low quality.
The area under coriander cultivation in Uttar Pradesh is 7800 ha with the production of 3580 million tons. The highest area under cultivation is Rajasthan with an area of 2, 14, 365 ha with the production of 2, 11, 943 million tons (Spice Board 2016).
Keeping in the view the use of bioagents, Neem oil and their combinations were tested on control of Alternaria blight. Calibre @ 0.2% (Meena *et al.*,2013) [8], *Trichoderma viride* @5% (Imtiaj *et al.*, 2008) [5], *Pseudomonas fluorescens* @5% (Singh *et al.*,2000), Neem oil @5% (Bochalya *et al.*, 2012), *Trichoderma viride*+ *Pseudomonas fluorescens* @5% (Kar and Sahu 2008) *Trichoderma viride* + Neem oil @5% (Bagwan 2010), *Pseudomonas fluorescens* + Neem oil @5% (Maheswaran 2003).

2. Materials and Methods
The present investigation on *Alternaria alternata* causing Alternaria blight of coriander was carried out in the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology & Sciences Allahabad. The coriander (*Coriandrum sativum*.) variety kushboo susceptible to Alternaria blight was used for investigation.
Isolation and Collection of diseased sample
Leaves were collected from infected plants bearing characteristic symptoms of concentric rings of Alternaria blight. These leaves symptoms after mounting on slide were examined under microscope to confirm the presence of *Alternaria sp.*
The infected leaf parts were cut into small pieces of two to three mm dimension in a manner so that pieces may have some green portion also. Such leaf bits were surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution for 30 seconds and washed three times with sterile

distilled water to remove any traces of mercuric chloride adhered with leaf bits. 2-3 leaf bits were transferred on PDA medium contained in Petri plates aseptically with the help of sterilized forceps.

These Petri plates were incubated at 25 ± 2˚C. After 3 days mycelia growth was observed around leaf bits from this colony growth, a portion from the periphery having single hyphal tip were separated and transferred to other Petri plates having medium to get pure culture and identification of the pathogen was confirmed by observing the morphological features of colony, spore characteristics.

2.1 Dual Culture Technique

Twenty ml of sterilized PDA was plated in 9 cm petri plates and allowed to solidify. Mycelial discs of 5mm diameter of the antagonists as well as the test pathogen were cut with sterile Cork borer from the periphery of an actively growing three day old cultures and then placed on opposite sides of petri plate. The distance between inoculums blocks was 7cm. The inoculated petri plates were incubated at 28±2^{0}C for three days.

The petri plates isolated with pathogen alone served as control. Three replications were maintained per treatment. The percent reduction in radial growth and reduction of mycelial growth in petri plates test pathogen was calculated by using the following formula. (Vincent 1927)

I = R1-R2/R1×100

Where

R_1, Radius of *A. alternata* colony in control plate;

R_2, Radius of *A. alternata* colony in dual culture plate.

2.2 Food Poisoning Technique

Twenty ml of sterilized PDA was added with required or prescribed dosage of botanicals and chemicals added along with PDA and was plated in 9 cm petri plates and allowed to solidify. Mycelial discs of 5mm diameter of the pathogen discs are placed were incubated at 28±2^{0}C for three days. The petri plates isolated with pathogen alone served as control. Three replications were maintained per treatment. The percent reduction in radial growth and reduction of mycelial growth in petri plates test pathogen was calculated by using the following formula. (Vincent 1927**)**

I= R1-R2/R1×100

Where

R1, Radius of *A. alternata* colony in control plate;

R_2, Radius of *A. alternata* colony in food poison plate.

Table 1: Details of the disease rating scale

Per cent all above ground parts infected	Score
Free from disease	0
1 to 10% area of leaf and umbel blighted	1
11 to 20% area of leaf, stem and umbel blighted	3
21 to 35% area of leaf, stem and umbel blighted	5
36 to 60% area of leaf, stem and umbel blighted	7
More than 61% area of leaf, stem and umbel blighted	9

The per cent disease intensity was calculated by using the formula of Wheeler (1969) was calculated a

$$PDI = \frac{\text{Sum of individual ratings}}{\text{Number of leaves observed x Maximum disease rati}} \times 100$$

3. Results

Result showed that the maximum disease intensity (%) was recorded in control (82.42) and the minimum disease intensity was observed in Manzeb (mancozeb +Carbendazim) @ 0.2%/l (32.14), followed by *Trichoderma viride*+ *Pseudomonas fluorescens* @ 5%/l (43.73), *Trichoderma viride* @ 5%/l (47.17), *Trichoderma viridae* + Neem oil @ 5% (55.61), *Pseudomonas fluorescens @5%/l* (46.61), Neem oil@ 5%/l (64.50) and *Pseudomonas fluorescens*+ Neem oil @ 5%/l (62.72). However, *Trichoderma viride* @ 5%/l + *Pseudomonas fluorescens* @5%/l showed that significant decrease in disease intensity compared to other fungicide.

Table 2: Effect of treatments on blight intensity at 60, 75 and 90 DAS of Coriander

S n	Treatments	60 das	75 das	90das
T0	Control	40.17	59.16	82.45
T1	Calibre	20.48	26.11	32.60
T2	Trichoderma viride	30.54	40.09	47.53
T3	*Pseudomonas fluorescens*	33.07	43.49	46.66
T4	Neem oil	38.16	50.27	64.32
T5	Trichoderma viride + Pseudomonas fluorescens	27.98	34.27	43.55
T6	Trichoderma viride +Neem oil	36.65	41.12	55.29
T7	Pseudomonas fluorescens + Neem oil	38.74	45.44	62.13
	Overall Mean	33.22	42.49	55.29
	F-test	S	S	S
	S. Ed.(±)	0.525	1.115	0.802
	C.D.(P=0.05)	1.135	2.386	2.076

Table 3: Effect of treatments on radial growth of Alternaria alternata.

S.N0	Treatments	Radial growth(mm)	% 0f inhibition
T0	Control	5.2	0.00
T1	Calibre	0.67	89.33
T2	*Trichoderma viride*	1.26	77.42
T3	*Pseudomonas fluorescens*	2.8	62.81
T4	Neem oil	3.8	52.00
T5	*Pseudomonas fluorescens*	1.08	79.21
T6	*Trichoderma viride* + Neem oil	1.9	71.03
T7	*Pseudomonas fluorescens* + Neem oil	3.2	58.00

	Overall Mean	2.488	-
	F-test	S	-
	S. Ed.(±)	0.102	-
	C.D.(P=0.05)	0.205	-

The maximum mycelia growth was recorded in the control T0-5.2mm,whereas the minimum growth was recorded in the chemical treatment T1-0.67mm,the growth of the mycelia in the rest of the following treatments was as follows *Trichoderma viride* T2-1.26mm,*Pseudomonas fluorescens* T3-2.8mm, Neem oil at 3.8mm, T4-2.8mm,then after the chemical the combination of the bioagents shows the maximum inhibition of the mycelia growth T5-*Trichoderma viride* + *Pseudomonas fluorescens* -1.08,the treatment T6 - *Trichoderma viride*+ Neem oil - 1.9 mm Mycelial growth, the final treatment T7 –*Pseudomonas fluorescens* + Neem oil-shows the 3.2 mm.

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