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Efficacy of Bio-agents and Neem Oil against *Alternaria porri* (Ellis) in Onion (*Allium cepa* L.)

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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

Onion (*Allium cepa* L.) is one of the important cultivated vegetable crops and can be grown in temperate, tropical and sub-tropical climates. It is the important vegetable and spice crop and known as "Queen of the kitchen". Area, Production and Productivity on onion in India (2020-21) was 16.54 million ha, 26.92 million Tones and 19.40 t/ha. There are many diseases and pests affecting onion. Among the diseases, majorly purple blotch, downy mildew, Stemphylium blight and basal rot attacks the onion and among the pests, onion thrips, red spider mite, onion fly attacks the onion. Among them, purple blotch of onion is one of the most severe diseases, which is caused by *Alternaria porri* which causes heavy losses in the onion. The treatments *viz., Trichoderma viride, Pseudomonas fluorescens, Bacillus subtilis* and neem oil were tested under field conditions against *Alternaria porri* during *Zaid* for their efficacy against the growth and disease parameters and an untreated plot will serve as control. At desired interval of days (45,60,75 DAT) readings were taken for growth parameters and disease intensity. Based on single trail, it was observed that among all treatments, seedling treatment with *Trichoderma viride* + *Pseudomonas fluorescens* + neem oil @ 5% showed the most significant results with maximum growth parameters and minimum disease intensity results followed by *Trichoderma viride* + *Bacillus subtilis* + neem oil @ 5% and the minimum growth parameters and maximum disease intensity was shown in untreated (control) plot.

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1. INTRODUCTION

Onion (*Allium cepa* L.) is one of the important vegetable crops belongs to family Amaryllidaceae and genus *Allium* with about more than 780 species usually perennial bulbous plants grown in worldwide [1]. It is widely cultivated secondly to tomato. Onion is also called as "Queen of kitchen". Onion is supposed to have its origin in the Middle East Asian countries and introduced in India from Palestine [2]. India is the second most important producer of onion in the world [3]. The major onion growing countries are China, India, USA, Egypt, Iran, Turkey and Pakistan. In India, it is mainly grown in Maharashtra, Andhra Pradesh, Karnataka, Madhya Pradesh and Uttar Pradesh. Onion as vegetable and spice it is used both as tender and mature bulb. Onion (*Allium cepa* L.) is one of the important condiments widely used in all households all the year round. In bulb group vegetables the most important crop is onion. The optimum temperature for the bulb production was 16-25 degree Celsius and with 70% relative humidity. The average pH of soil is around 5.5. The bulb is composed of concentric, fleshy, enlarged leaf base or scales. The outer leaf bases lose moisture, scaly and the inner leaves generally thicken as bulbs develop. Onion is consumed throughout the year by almost all peoples from rural as well as urban area. It has medicinal properties and dietary value like blood pressure diabetics, increasing blood fat can be controlled and it is also regarding as anticancer foodstuff [4].

Purple blotch which is the most dreaded disease in onion caused by *Alternaria porri* is a major production constraint for the successful cultivation of the onion crop in India. In India, the purple blotch disease was first reported in the Bombay State and stated *Macrosporium* sp. as causal agent [5]. Later the organism was identified as *Alternaria porri* [6]. *Alternaria porri* is an air-borne pathogen belonging to the sub-division Deuteromycotina. It is one the severe pathogen over onion and garlic crops. High relative humidity (80-90%) and optimum temperature (24 ± 10° C) favors for further development of purple blotch disease and causing considerable yield losses. About 50 -100 percent yield loss reported due to purple blotch disease [7].

The first symptoms are numerous tiny, white, circular or irregular spots, less than one millimeter in diameter. These gradually increase in size until in advanced stages the diseased areas cover several square cms over surface. As the spots increase in size, they become oval-shaped or irregular and the white color eventually changes to violet. Later stages of development show the central portion of the spots changing to purple, immediately surrounded by a pale-yellow orange to salmon band beyond which is a pale green zone. Dull violet-black zones within the lesions are also observed. The dark purple color is the most distinctive symptom of the disease [8]. *Alternaria porri* is seed-borne and soil inhabiting and spreads mainly through air borne spores. Spore and mycelium in diseased plant debris also serve as means of perennation. In general, the control of purple blotch disease of onion has been exclusively based on the application of hazardous chemicals like fungicides. Several effective fungicides have been recommended against this pathogen, but they are considered as a long- term solution, due to concerns of expense, exposure risks and the hazards of its residues. Moreover, the development of resistance of pathogenic fungi towards synthetic pesticides is of great concern that can affect significantly the efficacy of chemical fungicides. Thus, to find safe efficacious and environmentally friendly fungicides considered as source of major concern. The present study was aimed to determine the efficacies of different bio-agents and neem oil against purple blotch of onion under Prayagraj Agro-climatic conditions as this disease causes enormous losses to the farmers.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted at Central Research Field of Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj during *Zaid* season 2020-2021. The soil type of experimental site was sandy loam, low in organic carbon, nitrogen and phosphorus with the pH of 5.6. The experimental field was laid out in Randomized Block Design with 6 treatments and 3 replications.

2.2 Methodology

2.2.1 Collection of disease samples

Infected plant showing typical symptoms of disease, in the field of standing crops i.e., infected leaves of onion is selected. These collected disease samples were brought to the lab for furtherinvestigation.

2.2.2 Isolation and Identification of fungus

The infected leaf parts were cut into small pieces (2-3mm), surface sterilization was done with 0.1% sodium hypochlorite solution for 30 seconds, washed 3 times in sterilized distilled water and then transferred aseptically on petri dishes and test tubes containing PDA. Inoculated Petri plates were incubated at 27±1℃ for 5 to 7 days and examined at frequent intervals to see the growth. After a few days (3-4 days) the colony of the fungus appeared on the medium, the slide was prepared and identification of the pathogen Observation of the fungal colony characteristics was gone through microscopic examination. Using a sterile needle, small portion of disease infected sample were taken and placed on a sterile glass slide. It was stained by using lactophenol, cotton blue and covered with cover slip. Then the microscope was used for the observation of morphological characteristics of fungal structures [9].

2.2.3 Morphological characteristics of *Alternaria porri*

Conidiophores arising singly or in small groups, pale to mid brown. Conidia single, straight or slightly curved, obclavate or with body of conidium ellipsoidal and tapering to a beak, 8–12 transverse septa, 0–7 longitudinal or oblique septa, sometimes slightly constricted at septa, beak is often about same length as body of conidium. It is sometimes branched, pale colored,2–4 µm thick, tapering [10].

2.2.4 Evaluation of bio-agents and neem oil *in vivo*

The efficacy of bio-agents and neem oil against *Alternaria porri* was carried out *in vivo* conditions.

2.2.5 Disease intensity (%)

Disease intensity (%) formula given by wheeler [11] was used for calculation.

Disease intensity (%) = (Sum of all disease ratings/ Total no. of ratings X Maximum disease grade) X 100

All the leaves of selected plants were scored individually by following 0-5 scale [12].

2.2.6 Observations recorded

Pre-harvest observations were recorded during the experiment. They include Length of the flowering stem (cm), No. of leaves and Disease intensity (%).

3. RESULTS

3.1 Effect of Different Bio-agents and Neem Oil on Plant Growth Parameters

3.1.1 Length of the flowering stem

Observations of plant growth parameters like Length of the flowering stem (cm) were recorded at 45, 60 and 75 DAT of onion. The statistical analysis of data showed that all the treatments were found significantly effective. Results showed that at 75 DAT, the maximum length of the flowering stem was recorded in T4 – *Trichoderma viride* + *Pseudomonas fluorescens* + neem oil (54.933cm) followed by T5 – *Trichoderma viride* + *Bacillus subtilis* + neem oil (50.440cm), T3 - *Trichoderma viride* + *Pseudomonas fluorescens (48.773),*

Score/Grade	Disease description
0	No Disease symptom on leaf
1	Small light brown spots scattered covering ≤5% Leaf area
2	Spots small, brown, with concentric rings, covering 5.1 to 10% Leaf area
3	Spots large, brown, irregular, with concentric rings, covering 10.1 to 25% leafarea
4	Large, brown, irregular lesions with typical blight symptoms, covering 25.1 to50% Leaf area
5	Large, brown, irregular lesions with typical blight symptoms, covering morethan 50% leaf area

T_1 - *Trichoderma viride* + *Bacillus subtilis* (46.487cm), T_2 - *Pseudomonas fluorescens* + *Bacillus subtilis* (45.920cm) are effective over the length of the flowering stem as compared to T_0 - control (40.633cm). Among the treatments, all the treatments are statistically significant over control, the treatments (T_2 , T_1) are found non-significant to each other at 75 DAT [Table 1].

3.1.2 No. of leaves

Observations of plant growth parameters like No. of leaves were recorded at 45, 60 and 75 DAT of onion. The statistical analysis of data showed that all the treatments were found significantly effective. Results showed that at 75 DAT, the maximum no. of leaves was recorded in T_4 – *Trichoderma viride* + *Pseudomonas fluorescens* + neem oil (12.600) followed by T_5 – *Trichoderma viride* + *Bacillus subtilis* + neem oil (11.733), T_3 - *Trichoderma viride* + *Pseudomonas fluorescens* (10.600), T_1 - *Trichoderma viride* + *Bacillus subtilis* (10.133), T_2 - *Pseudomonas fluorescens* + *Bacillus subtilis* (9.533) are effective over the no. of leaves as compared to T_0 - control (8.400). Among the treatments, all the treatments are statistically significant over control, the treatments (T_1, T_3) are found non-significant to each other at 75 DAT [Table 1].

3.2 Disease Intensity (%)

Observations of disease intensity (%) was recorded at 45, 60 and 75 DAT of onion. The statistical analysis of data showed that all the treatments were found significantly effective. Results showed that at 75 DAT, the minimum percentage of disease intensity (%) was recorded in T_4 – *Trichoderma viride* + *Pseudomonas fluorescens* + neem oil (24.726) followed by T_5 – *Trichoderma viride* + *Bacillus subtilis* + neem oil (25.685), T_3 - *Trichoderma viride* + *Pseudomonas fluorescens* (28.825), T_1 - *Trichoderma viride* + *Bacillus subtilis* (31.985), T_2 - *Pseudomonas fluorescens* + *Bacillus subtilis* (36.146) are effective over the percentage of disease intensity (%) as compared to T_0 - control (52.136). However, the combination of the treatment *Trichoderma viride* + *Pseudomonas fluorescens* + neem oil shows the significant decrease in disease intensity over control. Among the treatments, all the treatments are statistically significant over control, the treatments (T_4, T_5) are found non-significant to each other at 75 DAT [Table 2].

Table 1. Effect of treatments on the length of the flowering stem (cm) and no. of leaves.

Treatments		Length of the floweringstem (cm)			No. of leaves		
		45 DAT	60 DAT	75 DAT	45 DAT	60 DAT	75 DAT
T_0	Control	26.453	35.540	40.633	2.600	4.933	8.400
T_1	*Trichoderma viride* + *Bacillus subtilis*	35.853	46.907	46.487	3.267	6.133	10.133
T_2	*Pseudomonas fluorescens* + *Bacillus subtilis*	32.247	43.567	45.920	3.067	5.733	9.533
T_3	*Trichoderma viride* + *Pseudomonas fluorescens*	40.400	47.480	48.773	3.667	6.667	10.600
T_4	*Trichoderma viride* + *Pseudomonas fluorescens* + Neem oil	46.527	54.600	54.933	4.600	8.200	12.600
T_5	Neem oil + *Trichodermaviride* + *Bacillus subtilis*	41.080	49.673	50.440	3.933	7.400	11.733
C. D. (0.05)		0.682	0.877	0.668	0.373	0.465	0.490
S. Ed (±)		0.302	0.388	0.296	0.165	0.206	0.217

Table 2. Effect of treatments on disease Intensity (%)

Treatments		Disease intensity (%)		
		45 DAT	**60 DAT**	**75 DAT**
T0	Control	24.552	40.761	52.136
T1	*Trichoderma viride + Bacillussubtilis*	18.665	24.971	31.985
T2	*Pseudomonas fluorescens +Bacillus subtilis*	.20.517	27.315	36.146
T3	*Trichoderma viride + Pseudomonas fluorescens*	17.997	22.632	28.825
T4	*Trichoderma viride + Pseudomonas fluorescens* + Neemoil	13.443	19.050	24.726
T5	Neem oil + *Trichoderma viride + Bacillus subtilis*	15.757	20.526	25.685
C. D. (0.05)		**1.146**	**1.739**	**1.406**
S. Ed (±)		**0.508**	**0.771**	**0.623**

Fig. 1. Symptoms on leaves

Fig. 2. Microscopic view of *Alternaria porri*

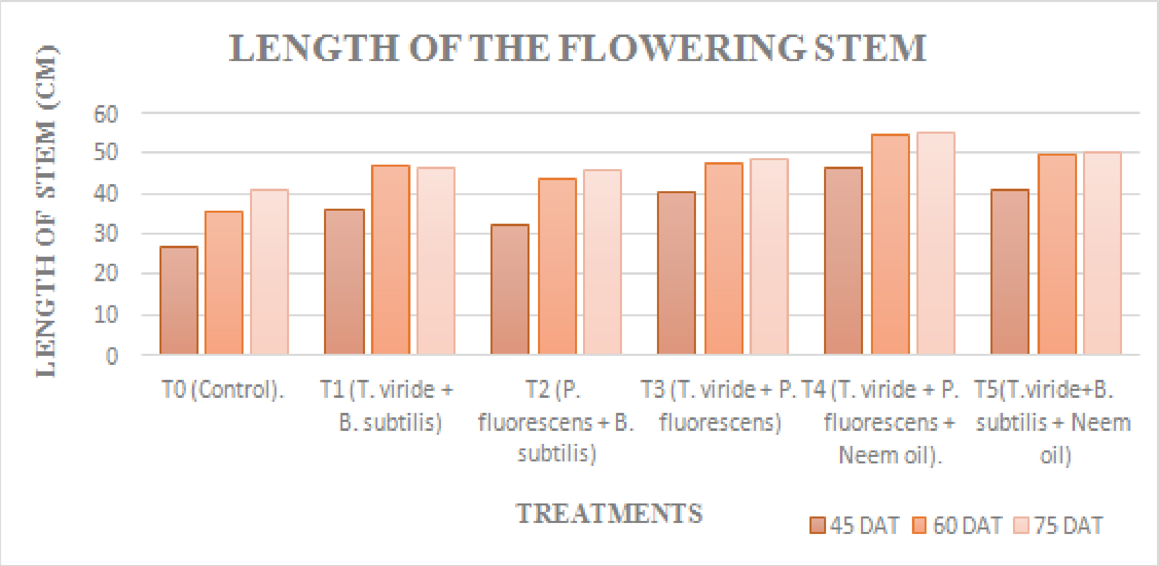

Fig. 3. Effect of treatments on the length of the flowering stem (cm)

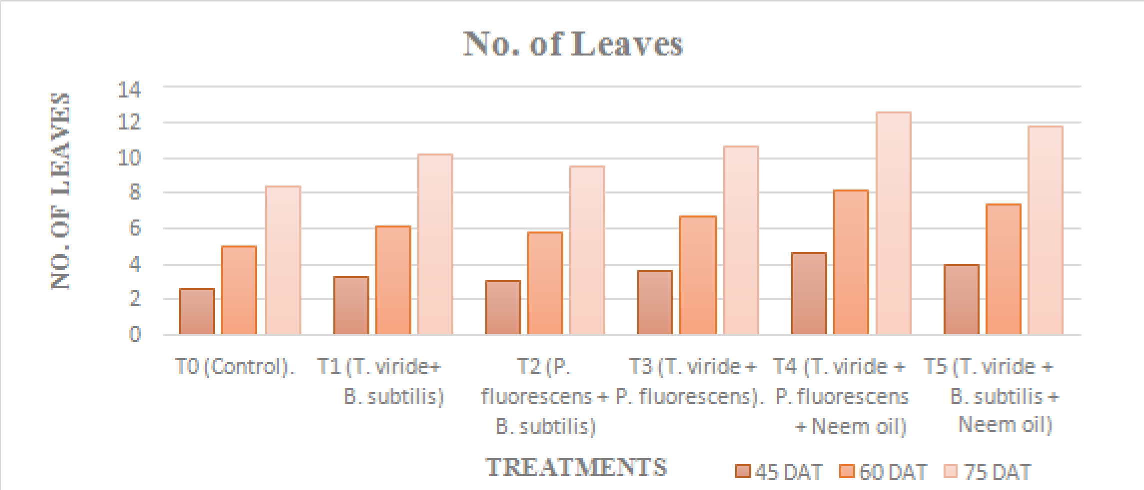

Fig. 4. Effect of treatments on the No. of Leaves

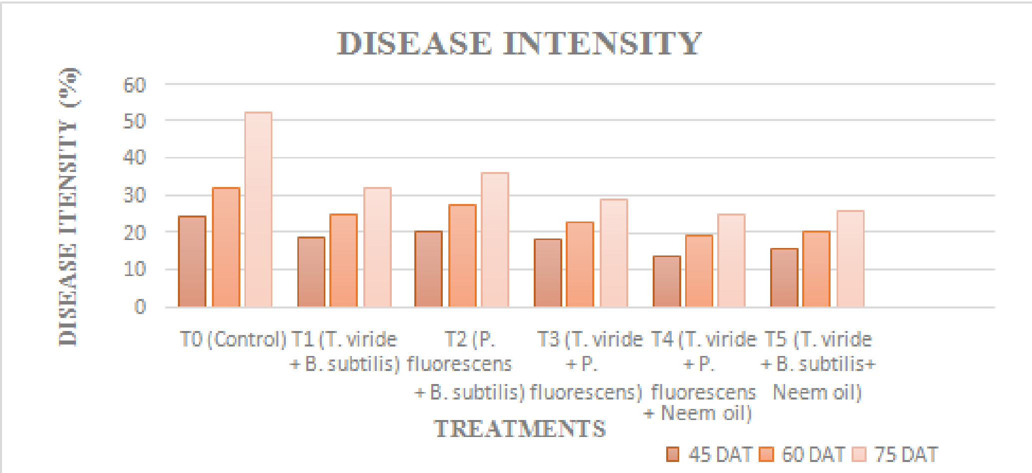

Fig. 5. Effect of treatments on the disease intensity (%)

4. DISCUSSION

The results in increased length of the flowering stem and no. of leaves were observed highest at the combination of T4 – *Trichoderma viride* + *Pseudomonas fluorescens* + neem oil. Similar findings were reported by Metwally and Al-Amri [13] and Prakasam and Pratibha [14]. They evaluated the efficacy of biocontrol agents used (alone or in various combinations) against *Alternaria spp*. and promoting plant growth.

Maximum length of the flowering stem and no. of leaves was observed in the combination of Trichoderma viride + Pseudomonas fluorescens + neem oil because inhibitory effect of bio-agents due to hyper parasitism/ mycoparasitism, competition for space and nutritional source and antagonistic chemical produced by them and the components present in the neem oil are responsible for the significant increase in growth of the plants. Inhibition of emergence of fungal pathogen due to existence of major components such as Azadirachtin, Saladin, nimbin, nimonol and iso-meldenin which has ability to disrupt the cell membrane and facilitate intracellular compounds leakages leads to cell lysis.

The present research findings were obtained and the comparison of the present investigation with the previous findings with various research reveals that the treatment combinations of Trichoderma viride + Pseudomonas fluorescens + neem oil shows the minimum percentage of disease intensity (%).

Similar results were also found in onion by Kumar and Biswas [15], Kumar and Palakshappa [16] , Ramjegathesh et al. [17] and Priyanka et al. [18] who reported that seedling treatment using T. viride and P. fluorescens can be used under field conditions to control purple blotch in onion. The use of the neem oil as a control of Alternaria spp. possess superiority over traditional usage of agrochemicals, such as the low cost of production, fast degradation, low toxicity in the environment and lack of health risks to producers and final consumers. It has an ability to reduce or inhibit the growth and development of Alternaria porri along with being economical and eco-friendly makes the neem oil suitable factor for development of a natural fungicide, which can retard the use of chemical fungicides.

The probable reason may be species of the genus *Trichoderma viride* possess many qualities and they have great potential use in agriculture such as amend 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 and mycoparasitism which includes, chemotropic growth of *Trichoderma viride*, recognition of the host by the mycoparasites, secretion of extra cellular enzymes, penetrations of the hyphae and lysis of the host.

Pseudomonas fluorescens embodies an attractive bio-control agent because of their catabolic adaptability, their outstanding root-colonizing abilities, and their capacity to produce a wide range of antifungal metabolites. Neem oil contains at least 100 biologically active compounds. Among them, the major constituents are triterpenes known as limonoids, the most important being azadirachtin, which appears to cause 90% of the effect on most pests. Other components present include meliantriol, nimbin, nimbidin, nimbinin, nimbolides, fatty acids (oleic, stearic, and palmitic), and salannin. It works by preventing the germination and penetration of fungal spores into leaf tissue.

5. CONCLUSIONS

Purple blotch of onion is a destructive disease that causes higher yield losses in onion. From the above results and summary, it has been concluded that, the *in vivo* results revealed that length of the flowering stem (cm), no. of leaves at 45, 60 and 75 DAT had found significantly increased and disease intensity (%) in onion at 45, 60 and 75 DAT significantly decreased in T4 - *T. viride*+ *P. fluorescens* + Neem oil @ 5%. These combinations of the treatments show most effective result in all the parameters. Therefore, it may be recommended for the better management of purple blotch of onion. The findings of the present experiment are limited to one crop season (*Zaid* - 2021) under Prayagraj agro-climatic conditions, as such to validate the present findings more such trials should be carried out in future.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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