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Integrated Effect of *Trichoderma harzianum* with Selected Botanicals Extracts on Alternaria Leaf Spot of Broccoli (*Brassica oleracea* var. *italica*) Caused by *Alternaria brassicae* (Berk.) Sacc.

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Broccoli (*Brassicae oleracea* var. *italica*) is an important cole crop vegetable, belong to family Brassicaceae. Alternaria leaf spot caused by *Alternaria brassicae* is one of the serious diseases in broccoli. *In vitro* and *in vivo* experiment were conducted at the research plot of the Department of

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Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh in *Rabi* season 2023-2024, to evaluate *Trichoderma harzianum* and botanicals on Alternaria leaf spot of broccoli caused by *A.brassicae*. *In vitro* condition, five botanical extracts were taken at the concentration 10% and *T. harzianum* at 10% and Mancozeb at 0.2% (treated check). *In vivo* condition, eight treatments were taken and all seedlings were treated with *T. harzianum* at 10%, Mancozeb at 0.2% and foliar spray of botanical extracts @10% except T0– control (untreated check) and (treated check). *In vitro* condition, among the treatments Neem leaf extract at 10% were observed effective in the per cent disease inhibition (%) (79.77) followed by Eucalyptus leaf extract at 10% (75.69). Among the treatments, under *In vivo* condition, T3- *T. harzianum* (S.T.) + Neem leaf extract (F.S.) was observed minimum disease intensity (16.59, 19.85 and 23.28 %) at 45,60 and 75 DAT, respectively. Maximum plant height (9.78, 23.84 and 36.66cm) at 30,60 and 90 DAT, number of leaves per plant (9.36, 17.26 and 28.00), head weight (0.38 kg) and yield (9.68 t/ha) were found in T3- *T. harzianum* (S.T.) and Neem leaf extract (F.S.) when compared to treated T7- mancozeb (treated check) and T0- Control (untreated check).

Keywords: Alternaria leaf spot; Alternaria brassicae; botanicals plant extracts; broccoli; Trichoderma harzianum.

1. INTRODUCTION

"Broccoli (*Brassica oleracea* var. *italica*) is an important cole crop vegetable, belong to Brassicaceae family originated from Italy about more than 2000 years ago [1]. Broccoli is native to the eastern Mediterranean and Asia minor, broccoli was cultivated in Italy in ancient roman times. It was introduced to England in the 18th century and became popular in the United States in the 20th century" [2]. "The common English name broccoli' is derived from the Italian word 'broccolo', meaning 'the flowering crest of a cabbage'. Fresh broccoli is dark green in colour with firm stalks and compact bud clusters. As a vegetable, it is grown for its edible flower buds and stalk" [3].

"Broccoli is a biennial plant belonging to the Brassicaceae family that is eaten as a vegetable throughout the world. The edible plant parts are the stalk and large flowering head. Broccoli is a rich source of vitamin C and vitamin K. Broccoli became one of the favourite foods due to its high nutrient and fiber content. Broccoli also contains numerous phytochemicals, such as polyphenols, namely kaempferol, quercetin glucosides, isorhamnetin, glucosinolates, and their derivatives. These are responsible for its antioxidant and anticancer properties and other health benefits" [4].

"Broccoli is considered a cool season crop, which has now been distributed to both tropical and subtropical areas" [5]. "This crop can be cultivated in various parts of India during the winter season, especially in regions where rainfall is not excessive. Areas with moderate rainfall and cooler temperatures are generally suitable for broccoli farming. Being a cool season crop, it requires 15-20° C temperature for head creation. Temperature above 25° C isn't favourable for its development and can cause slackening and darting of heads" [6].

"The global production of broccoli and cauliflower was 25,531,274 tons from an area of 1,357,186 ha, in which Europe ranks third (after China and India) and contributes 9.5 and 5410.5% in production and area, respectively" [7]. "This vegetable, closely resembling cauliflower but usually green in color, introduced in India many years after cabbage and cauliflower and has gained popularity in short span of time. Now, India stands at second position for cauliflower and broccoli production with an annual production of 6.7 million tonnes" [8]. "It is mostly cultivated in hilly areas of Himachal Pradesh, Uttar Pradesh, Jammu and Kashmir, Nilgiri Hills and Northern plains of India" [9].

"There are many diseases and pests collectively that lower the yield and quality of broccoli crops. Broccoli is infected by various types of pathogens including bacteria, fungi, and viruses, which lead to reduction of yield and quality" [10]. "Among all the diseases Alternaria leaf spot disease caused by Alternaria sp. is the most important major disease which resulted significant decline in the yield of all cultivars" [11].

"Among the different diseases caused by the genus Alternaria, Alternaria leaf spot disease is most dominant one that causes average yield loss in the range of 32-57%" [12]. "During 2017

and 2018, severe symptoms of dark spots on leaves were observed on broccoli plants (Brassica oleracea L. var. italica) cultivar with 45 to 37% disease incidence and 70% yield loss" [13].

For the management of this disease fungicides have been recommended to manage the disease, but present-day farmer perceptions and environmental hazards are compelling to search for alternative eco-friendly disease management strategies [14]. So this situation compels to focus on disease management by utilizing biological agents, plant extracts and fungicides in lowest concentration. Application of biological agents and extract is eco-friendly and a sustainable approach apart from being a promising alternative to fungicide application. In the present study among the plant extracts, Lantana camara was found to be effective in managing the Alternaria blight infection in the field (71.92% reduction in disease severity and 68.18% increase in yield) in comparison to bio-agent (Trichoderma viride) 36.27% reduction in disease severity respectively [15].

2. MATERIALS AND METHODS

The experiment was conducted at the research plot of the Department of Plant Pathology, Central Research Field, Sam Higginbottom University of Agriculture, Techonology and Sciences, Prayagraj during the *Rabi* season of 2023-2024. Field experiment was laid out in Randomized Block Design with eight treatments having three replications.

2.1 Isolation and Identification of Pathogen

The leaves were collected from infected plants bearing characteristic symptoms of Alternaria leaf spot of broccoli. The leaves were thoroughly washed under running tap water. The symptoms on leaves after mounting on slide were examined under microscope to confirm the presence of *Alternaria* sp. The infected leaf parts along with the healthy portion was cut into small pieces under aseptic conditions and surface sterilized with 1% sodium hypo chlorite (NaOCl) solution for 1 minute and washed three times with sterile distilled water to remove any traces of sodium hypo chlorite (NaOCl) adhered with leaf bits. Then they were placed on filter paper so that extra water gets absorbed. After that five leaf bits were transferred on PDA media [16] contained in sterilized petri plates with the help of forceps. To avoid bacterial contamination streptomycin @ 100 ppm, was added in the medium at lukewarm stage before pouring PDA into Petri plates. Then Petri plates were wrapped and incubated at 27±2°C in BOD, after 3 days mycelia growth was observed around leaf bits. With the help of cork borer a portion from the periphery having single hyphal tip from this colony growth was separated and transferred to other petri plates having medium to get pure culture and identification of the pathogen was recorded by observing the morphological features of colony, spore characteristics and referring the relevant literature [17].

2.2 Evaluation of botanical extracts and *Trichoderma harzianum* against Alternaria leaf spot of broccoli

2.2.1 *In vivo* evaluation

Botanical extracts were prepared using method of standard procedure given by Mahapatra and Das [18]. Matured leaves and other botanicals were collected and sterilized with distilled water, the leaves were homogenized in a pestle and mortar, sterilized distilled water. Aqueous extract of this botanical (1% w/v) were prepared by mixing 100g fresh leaves / botanicals of plant with 100 ml of sterile distilled water and crushing in warring blender. The extract was filtered through four layers of moisture muslin cloth. The filtrate thus obtained was considered as stock solution. The phyto-extracts were sprayed @10% prepared from standard solution, Mancozeb at a ratio 0.2%, fungal bio-agent *T. harzianum* were also sprayed @10% after the appearance of the first symptoms in the field at thirty days after transplanting (DAT), the foliar spray of botanicals, mancozeb and *T. harzianum* was applied three times at fifteen days interval. Unsprayed plots were kept as control.

2.2.2 Disease intensity

The intensity of disease was visually assessed in all the plots at weekly interval from first appearance of disease for each treatment. Disease intensity was calculated using the formula given by Wheeler [19].

Disease Intensity (%) = Sum of all disease ratings / Total no of ratings × Maximum disease grade ×100

2.2.3 *In vitro* evaluation

2.2.3.1 Culture of T. harzianum

One g of product was taken and mixed in nine ml of sterilized distilled water in a clean and sterilize test tube to make 10-1 dilution (1:10) shaken well and one ml of the suspension of nine ml of sterilize water was taken in a tube to make 10-2 dilution (1:100). Similarly, four more serial dilution in the same way was done to get 10-6 dilution and transferred one ml of this suspension to sterile petri plate containing15-20ml of sterilized melted and cooled PDA media the plates were rotated gently and allowed to solidify. The petri plates were incubated in BOD incubator at 25 + 2 °C for five to seven days. The development of typical colony of *T. harzianum* was observed.

2.2.3.2 Preparation of plant extracts

The botanicals used for the experiment were Neem, Black night shade, Eucalyptus, Datura, Lantana. The botanical part used for the treatment were Neem leaf extract 10%, Black night shade leaf extract 10%, Eucalyptus leaf extract 10%, Datura leaf extract 10%, Lantana leaf extract 10%. Aqueous plant extracts were obtained as per the method described by Bhatti [20]. A 100-gram sample of each fresh leaves were gently wash under running tap water and again wash it in sterile distilled water. Then each sample was grind separately by using sterile pestle and mortal with 100 ml sterile distilled water. The extract of each sample thus obtained was filtered separately through a sterilized double layered muslin cloth to remove the bits of plant material is filtrate. Then extract was again filtered through a filter paper (Whatman No.1). This formed the standard plant extracts solution (100%). All the glassware used in the study were sterilized before use. All the plant extracts were tested at 10 per cent concentration against the test pathogen using PDA as a basal medium. To obtain 10 per cent concentration of plant extracts, 100 ml of lukewarm PDA was mixed with 10 ml of standard plant extracts in 250 ml conical flask, separately and then it was stirred well to obtain homogenized mixture.

2.2.4 Dual Culture Technique

Twenty ml of sterilized and cooled potato dextrose agar medium was poured into sterilized petri plates. Fungal antagonists were evaluated by inoculating a pathogen at one side of the petri plate and the antagonist at exactly opposite side of the same plate by leaving a space of 4 cm. After required period of incubation i.e., when the growth in control plate records 90 mm in diameter, the radial growth of the pathogens was measured [21].

Percentage inhibition of mycelia growth of test pathogen was calculated using the formula:

$$I = C – T /C \times 100$$

Where,

I = Percent reduction in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment

2.2.5 Poisoned food technique

Five mm diameter of culture disc of *A. brassicae* was be kept at the center of each petri plate containing the botanicals of required concentration dissolved in PDA. Three replications were maintained. The plates were incubated at 27±2˚C for seven days and colony diameter was recorded [21].

Per cent inhibition of mycelial growth was calculated by using the formula:

$$I = C – T /C \times 100$$

Where,

I = Percent inhibition
C = Growth (mm) of test fungus in untreated control plate
T = Growth (mm) of test fungus in treated plate

3. RESULTS AND DISCUSSION

3.1 Effect of Plant Extracts on *A. brassicae* by Poison Food Technique

3.1.1 Radial growth (mm) of *A. brassicae*

The data presented in Table 1, depicted reveal significant reduction in T3- Neem leaf extract (18.20mm) followed by T2 – Eucalyptus leaf extract (21.87mm), T5 – Black night shade leaf extract (23.83mm), T4– Lantana leaf extract (26.00mm), T1- Datura leaf extract (26.98mm) as compared to mancozeb (treated check)-(2.50mm) and untreated check control T0 -(90mm).

3.1.2 Percent inhibition of mycelia growth

The data present presented in Table 1 and Fig. 1 reveal that there was significant increase in T3- Neem leaf extract at 10% (79.77%) followed by T2 – Eucalyptus leaf extract at 10% (75.7%), T5 – Black night shade leaf extract at 10% (73.52%), T4 – Lantana leaf extract at 10% (71.11%), T1- Datura leaf extract at 10% (70.02%) as compared to T7-mancozeb (treated check) (97.21%) and untreated check control T0 - (0%). The probable reason for such findings may be due to the fact that Neem leaf contains active compounds such as azadirachtin as well as salanin, nimbim. Constituents such as nimbim, nimbidin, limonoids which disrupts the cell of the pathogen and restricts the performance of some enzymes that are important to proliferate fungi in nature and leads to their death. The current findings are consisting with the research conducted by Vijaykumar et al. [22] and Kumar et al. (2007) who tested the effectiveness of different phytoextracts against *A. brassicae* and found that the antifungal activity and potentiality of nimbicidin and Neem leaf extract have been proved successfully against the causal fungus.

3.2 Effect of Treatments on Disease Intensity of Alternaria Leaf Spot of Broccoli Caused by *A. brassicae*

The data presented in the Table (2) reveal that the disease intensity (%) on broccoli significantly reduced by T3 – *T. harzianum* + Neem leaf extract at 10% (23.28%) followed by T6-(*T. harzianum* at 10% (26.31%), T5 – (*T. harzianum* + Black night shade leaf extract at 10% (30.26%), T4- (*T. harzianum* + Lantana leaf extract at 10% (32.56%),T2 – *T. harzianum* + Eucalyptus leaf extract at 10% (36.83%), T1- (*T. harzianum* + Datura leaf extract at 10% (37.28%) as compared to the T7 –mancozeb at 0.2% (treated check) (18.96%) as well as (T0) – control (untreated check) (40.64%).Similar findings have been reported by Anshika and Zacharia [23] and Ravella et al. [24]. The probable reason for this result may be due to the Neem having anti- microbial activity. Neem has reported to exhibit antifungal, antibacterial and insecticidal properties. The ethanolic extract of Neem leaves stops the growth of fungi. *T. harzianum* has shown significant results on disease intensity and yield attributes, whereas yield has been significantly increased and decreased disease intensity which may be due to the mycoparasite nature and ability to generate volatile and non-volatile compounds against pathogen with great anti- microbial activity.

3.3 Effect of Treatments on Plant Height (cm) of Broccoli

The data presented in the Table (3) reveal that the plant height (cm) on broccoli significantly increased plant height in T3 –(*T. harzianum* + Neem leaf extract at 10% (36.66 cm) followed by T6-(*T. harzianum* at 10% (33.70 cm), T5 – (*T. harzianum* + Black night shade leaf extract @10% (31.10 cm), T2- (*T. harzianum*+ Eucalyptus leaf extract at 10% (29.93 cm), T4 – (*T. harzianum* + Lantana leaf extract at 10% (28.41 cm), T1- (*T. harzianum* + Datura leaf extract at 10% (27.78 cm) as compared to the T7 – Mancozeb at 0.2% (treated check)(39.47 cm) as well as (T0)– control (untreated check) (23.01 cm). The probable reason for this result may be due to Neem aqueous extract showed a promotive effect on shoot lengths, branches and leaves numbers. The bio-efficacy of Neem extract over pathogens can be attributed to the fact that Neem has active compounds such as azadirachtin, nimbin, nimbidin, nimbinene and

Table 1. Effect of selected plant extracts on radial growth (mm) of *A. brassicae*

Sr. No.	Treatments	Concentration	Mean colony diameter (mm)	Percent inhibition
T0	Control (untreated check)	-	90.00	0.00
T1	Datura leaf extract	10%	26.98a	70.02
T2	Eucalyptus leaf extract	10%	21.87	75.69
T3	Neem leaf extract	10%	18.20	79.77
T4	Lantana leaf extract	10%	26.00a	71.10
T5	Black night shade leaf extract	10%	23.83	73.51
T6	Mancozeb	0.2%	2.50	97.21
S.Em. (+)			0.51	0.57
C.D (5%			1.59	1.76

Fig. 1. Efficacy of plant extract on percent mycelial inhibition of *A. brassicae* by poisoned food technique

Table 2. Percent disease intensity (%) at 45, 60 and 75 DAT as affected by treatments

Sr. No.	Treatments	45DAT	60DAT	75DAT
T_0	Control (untreated check)	25.98	33.83	40.64
T_1	*T.harzianum* 10% (S.T.) + Daturaleaf extract 10%(F.S.)	23.40	29.88	37.28
T_2	*T.harzianum* 10% (S.T.) +Eucalyptus leaf extract 10%(F.S.)	22.50	29.55	36.83
T_3	*T. harzianum* 10% (S.T.) + Neem leaf extract 10%(F.S.)	16.59	19.85	23.28
T_4	*T. harzianum* 10% (S.T.) + Lantanaleaf extract 10%(F.S.)	21.34	27.51	32.56
T_5	*T. harzianum* 10% (S.T.) + Black night shade leaf extract 10%(F.S.)	20.28	25.64	30.26
T_6	*T. harzianum* 10% (S.T.) + *T. harzianum* 10%(F.S.)	18.26	22.36	26.31
T_7	Mancozeb (treated check) 0.2% (S.T. + F.S.)	11.66	15.90	18.96
S.Em. (+)		0.36	0.30	0.34
C.D (5%		1.09	0.92	1.03

Table 3. Effect of treatments on plant height (cm) of broccoli at 30,60 and 90 DAT

Sr. No.	Treatments	30DAT	60DAT	90DAT
T_0	Control (untreated check)	6.92	15.40	23.01
T_1	*T. harzianum* 10% (S.T.) + Datura leaf extract 10%(F.S.)	7.46	17.06	27.78
T_2	*T. harzianum* 10% (S.T.) + Eucalyptus leafextract 10%(F.S.)	8.96	19.16	29.93
T_3	*T. harzianum* 10% (S.T.) + Neem leaf extract 10%(F.S.)	9.78	23.84	36.66
T_4	*T. harzianum* 10% (S.T.) + Lantana leaf extract 10%(F.S.)	8.21	18.28	28.41

azadirone which are antifungal, anti-bacterial and anti-insecticidal in nature Nahak et al. [25] *Trichoderma* strains colonies plant roots, establishing chemical communication and systemically altering the expression of numerous plant genes that alter plant physiology. Application of *T. harzianum* significantly increased curd circumference length, the diameter of the stem Rosa et al. [26].

3.4 Effect of Treatments on Number of Leaves Per Plant of Broccoli

The data presented in Table (4) reveal that number of leaves significantly increased in T3 – (*T. harzianum* + Neem leaf extract at 10% (28.00) followed by T6- (T.harzianum at 10% (25.20), T5 – (*T. harzianum* + Black night shade leaf extract at 10% (23.40), T2- (*T. harzianum* +

Eucalyptus leaf extract at 10% (21.13), T4–(*T. harzianum* + Datura leaf extract at 10% (20.66), T1- (*T.harzianum* + Lantana leaf extract at 10% (20.40) as compared to the T7 – Mancozeb at 0.2% (treated check) (31.53) as well as (T0) control (untreated check) (16.00). The probable reason for this result may be due to Neem aqueous extract showed a promotive effect on shoot lengths, branches and leaves numbers. The bio-efficacy of Neem extract over pathogens can be attributed to the fact that Neem has active compounds such as azadirachtin, nimbin, nimbidin, nimbinene and azadirone which are antifungal, antibacterial and anti-insecticidal in nature Nahak et al. [25] Trichoderma strains colonise plant roots, establishing chemical communication and systemically altering the expression of numerous plant genes that alter plant physiology. Application of T. harzianum significantly increased curd circumference length, the diameter of the stem, and the leaf greenness index value Rosa et al. [26].

3.5 Effect of Treatments on Head Weight (kg) of Broccoli

The data presented in Table (5) and Fig. (2) reveal that head weight (kg) of broccoli significantly increased in T3 – *T. harzianum* + Neem leaf extract at 10% (0.38 kg) followed by T6- *T. harzianum* at 10% (0.35 kg), T5 – *T. harzianum* + Black night shade leaf extract at 10% (0.304 kg), T4- *T. harzianum* + Lantana leaf extract at 10% (0.301 kg), T2 – (*T. harzianum* + Eucalyptus at 10% (0.21), T1- *T. harzianum* + Datura leaf extract at 10% (0.20kg) as compared to the T7 – Mancozeb at 0.2% (treated check) (0.40 kg) as well as (T0) – control(untreated check) (0.16 kg). The probable reason for such findings may be that the Trichoderma and plant interaction produce the secondary metabolites such as auxin-like compounds or auxin- inducing substances that resulted in improved growth, head diameter and yield Tanwar et al. [27]. Neem extract is composed of antimicrobial ingredients such as alkaloids, glycosides, flavonoids, and saponins, which are common antibiotics found in plants. At concentrations of 0.1 and 0.4 gmL-1, Neem extract inhibited the growth of soil microorganisms Sarawaneeyaruk et al. [28].

3.6 Effect of Treatments on Yield (t/ha) of Broccoli

The data presented in Table (6) and Fig. (3) revealed that yield of broccoli significantly increased head weight T3 –*T. harzianum* + Neem leaf extract at 10% (9.68 t ha^{-1}) followed by T6- *T. harzianum* at 10% (7.92 t/ha), T5 – (*T. harzianum* + Black night shade leaf extract at 10% (6.81 t ha^{-1}), T4- (*T. harzianum* + Lantana leaf extract at 10% (6.50 t ha^{-1}), T4 –(*T. harzianum* + Eucalyptus leaf extract at 10% (6.19 t ha^{-1}), T1- (*T. harzianum* + Datura leaf extract @10% (5.68 t ha^{-1}) as compared to the T7 – Mancozeb at 0.2% (treated check) (11.55 t h^{-1}) as well as (T0) – control (untreated check) (3.85 t/ha). The probable reason for this result may be due to the production of secondary metabolites

Table 4. Effect of selected treatments on number of leaves per plant of broccoli at 30,60 and 90 DAT

Sr. No.	Treatments	30DAT	60DAT	90DAT
T_0	Control (untreated check)	7.73	10.46	16.00
T_1	*T. harzianum* 10% (S.T.) + Daturaleaf extract 10%(F.S.)	8.16	12.40	20.66
T_2	*T. harzianum* 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	8.93	13.40	21.13
T_3	*T. harzianum* 10% (S.T.) + Neem leaf extract10%(F.S.)	9.36	17.26	28.00
T_4	*T. harzianum* 10% (S.T.) + Lantanaleaf extract 10%(F.S.)	8.46	12.93	20.40
T_5	*T. harzianum* 10% (S.T.) + Black night shadeleaf extract 10%(F.S.)	8.86	14.16	23.40
T_6	*T. harzianum* 10% (S.T.) +*T. harzianum* 10%(F.S.)	9.76	15.73	25.20
T_7	Mancozeb (treated check) 0.2% (S.T. +F.S.)	10.13	20.13	31.53
S.Em. (+)		0.11	0.21	0.21
C.D (5%		0.33	0.66	0.64

such as antibiotics, isocyanide, acids and cell wall degrading enzymes which are implicated in the growth in the growth inhibition of many phytopathogenic fungi Supriya et al. [29,30] *T*. *viride* has shown significant results on yield attributes, the yield has been significantly increased which may be altributed to the mycoparasitic nature and ability to generate volatile and non-volatile compounds against pathogen with great anti-microbial activity [31,32].

Table 5. Effect of treatments on head weight (kg) of broccoli

Sr. No.	Treatments	Head weight / kg
T_0	Control (untreated check)	0.16
T_1	*T. harzianum* 10% (S.T.) + Datura leaf extract 10%(F.S.)	0.20
T_2	*T. harzianum* 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	0.21
T_3	*T. harzianum* 10% (S.T.) + Neem leaf extract 10%(F.S.)	0.38
T_4	*T. harzianum* 10% (S.T.) + Lantana leaf extract 10%(F.S.)	0.301
T_5	*T. harzianum* 10% (S.T.) + Black night shade leaf extract 10%(F.S.)	0.304
T_6	*T. harzianum* 10% (S.T.) + *T. harzianum* 10%(F.S.)	0.35
T_7	Mancozeb (treated check) 0.2% (S.T. +F.S.)	0.40
S.Em. (+)		0.05
C.D (5%		0.15

Table 6. Effect of treatments on total yield (t/ha) of broccoli

Sr. No.	Treatments	Yield (t ha^1)
T_0	Control (untreated check)	3.85
T_1	*T. harzianum* 10% (S.T.) + Datura leaf extract 10%(F.S.)	5.68
T_2	*T. harzianum* 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	6.19
T_3	*T. harzianum* 10% (S.T.) + Neem leaf extract 10%(F.S.)	9.73
T_4	*T. harzianum* 10% (S.T.) + Lantana leaf extract 10%(F.S.)	6.50
T_5	*T. harzianum* 10% (S.T.) + Black night shade leaf extract 10%(F.S.)	6.81
T_6	*T. harzianum* 10% (S.T.) + *T. harzianum* 10%(F.S.)	7.35
T_7	Mancozeb (treated check) 0.2% (S.T. +F.S.)	11.55
S.Em. (+)		0.12
C.D (5%		0.37

Fig. 2. Weighing of broccoli

Fig. 3. Yield of broccoli

4. CONCLUSION

It can be concluded that T. harzianum and botanicals Neem leaf extract is significant in controlling A. brassicae in broccoli. This study contributes valuable insights into sustainable disease management practices by the used of bio-control agent and plant extracts that can benefit both farmers and the agricultural sector. It is important to note that this investigation has been conducted in a specific cropping season within the agro-climatic conditions of Prayagraj. Further trials in diverse locations and seasons are recommended to expand upon these promising results.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Buck, P.A. Origin and taxonomy of broccoli. Economic Botany. (1956); 10:250-263.
2. Moreno DA, Lopez-Berenguer C, Carvajal M, Garcia-Viguera C. Health benefits of broccoli. Influence of pre-and post-harvest factors on bioactive 363 compounds. Food. 2007;1(2):297-312.
3. Poulomi Das, Dipanjan Ghosh. Yours Truly Broccoli. Science and Yours Truly Broccoli. Science and Culture. 2021; 1956;8(3-4).
4. Nagraj GS., Chouksey A, Jaiswal S, Jaiswal AK, Broccoli. In Nutritional composition and antioxidant properties of fruits and vegetables. Academic Press. 2020;5-17.
5. Sivakumar V, Srinivasulu A, Rao KM, Reddy RVSK. Performance of Broccoli (*Brassica oleracea* var. italica L.) genotypes under high altitude Tribal zone of Andra Pradesh. Emergent Life Science Research. 2022;8:84-88.
6. Kumar Sanjay, Kumar Pradeep, Meena ML, Kumar R, Rawat R, Yadav S. Influence of varieties and spacing on growth characters of sprouting broccoli (*Brassica oleracea* L.). Annals of Plant and Soil Research. 2021;23(1):99-103.
7. Siomos AS, Koularmanis K, Tsouvaltzis P. The impacts of the emerging climate change on broccoli (*Brassica oleracea* L. var. italica Plenck.) crop. 392 Horticulturae. 2022;8(11):1032.
8. Tejaswini T, Varma LR, Verma P, Prajapathi RI, Vani FB. Performance of different varieties with respect to growth, yield and quality of broccoli (*Brassica*

oleracea var. italica L.) under North Gujarat Conditions. International Journal of Current Microbiology and Applied Sciences. 2018; 12(11):3003-3009.
9. Meena M, Swapnil P, Zehra A, Dubey MK, Upadhyay RS. Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. Archives of Phytopathology and Plant Protection. 2020;50(13-14):629- 648.
10. Zhang X, Yao Y, Dhanasekaran S, Li J, Ngea GLN, Gu X, Zhang H. Controlling black spot of postharvest broccoli by *Meyerozyma guilliermondii* and its regulation on ROS metabolism of broccoli. Biological Control.2022;170:104938
11. Lafi OI, El-Hamarnah HA, Al-Saloul NJ, Radwan HI, Abu-Naser SS. A proposed expert system for broccoli diseases diagnosis. 2022;6(5):43-51.
12. Singh CK, Singh SP, Tiwari T, Singh. Bio-efficiency of plant extract, mancozeb and *Trichoderma viride* to control the Alternaria leaf spot of cabbage. Annals of Plant and Soil Research. 2015;17: 219-221.
13. De Britto S, Kapera D, Jogaiah, S. First report of leaf spot disease causedby *Alternaria brassicicola* on broccoli in Papua New Guinea. Plant Disease. 2020;104(11):3073- 3073.
14. Sakhare SS. Eco-friendly management of alternaria leaf spot of cabbage caused by *Alternaria brassicae* (berk.) sacc (Doctoral dissertation, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani); 2021.
15. Ahmad A, Ashraf Y. *In vitro* and *in Vivo* management of *Alternaria* leaf spot of Brassica campestris L. Plant Pathology & Microbiology. 2016;7:7
16. Tuite J. Plant Pathological Methods. Fungi and Bacteria. Burgess: Minneapolis. 1969;239.
17. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi.1972;132.
18. Mahapatra S, Das S. Bio efficacy of plant extracts against Alternaria leaf blight of mustard under field condition. Bioscan. 2013;8(2):675-679.
19. Wheeler BEJ. An introduction to plant diseases. An introduction to plant diseases; 1969.
20. Bhatti DS. Utilization of toxic plants for the control of nematode pests of economic crops: Final Technical Report April 1, 1983 to March 31, 1988. Haryana Agricultural University.
21. Vincent JM. Distortion of fungal hyphae in the presence of certain 414inhibitors. Nature. 1947;159(4051):850- 850.
22. Vijaykumar KN. Variability and integrated management of purple blotch of garlic (*Allium sativum* L.) caused by *Alternaria porri* Ellis Ciferri. M.Sc. Thesis, Department of Plant Pathology, University of Agricultural Sciences, Dharwad; 2022.
23. Anshika, Zacharia S. Biorational approaches for purple blotch disease of garlic (*Allium sativum L*.) incited by *Alternaria porri* (Ellis) Cif. International Journal of Environment and Climate Change. 2023;13(10):1612-1620.
24. Ravella, N. K., Udathala, D. and Iska, S. R. Efficacy of eco-friendly treatments on yield attributes in Indian mustard (Brassica juncea L.) against Alternaria blight. Environment Conservation Journal.2023; 24(1): 171-175.
25. Nahak G, Sahu RK. Biopesticidal effect of leaf extract of neem (*Azadirachta indica* A. Juss) on growth parameters and diseases of tomato. Journal of Applied 369 and Natural Science. 2015; 7(1): 482-488.
26. Rosa R, Franczuk J, Zaniewicz-Bajkowska A, Hajko L. Effects of *Trichoderma harzianum* and boron on spring broccoli. Applied Ecology & Environmental Research. 2019;17(2).
27. Tanwar A, Aggarwal A, Kaushish S, Chauhan S. Interactive effect of AM fungi with *Trichoderma viride* and *Pseudomonas fluorescens* on growth and yield of broccoli. Plant Protection Science. 2013;49(3):137-145.
28. Sarawaneeyaruk S, Krajangsang S, Pringsulaka O. The effects of neem extract and azadirachtin on soil microorganisms. Journal of Soil Science and plant nutrition. 2015;15(4):1071-1083.
29. Supriya V, Tiwari S, Sindu PL, Govardhani P. Effect of selected bio-agents and botanical on Alternarial leaf spot of coriander (*Coriandrum sativum* L.) caused by *Alternaria alternata* (Fries) Keissler. International Journal of Plant and 399 Soil Science. 2022;34(23): 1532-1537.
30. Dennis C, Webster J. Antagonistic properties of species-groups of Trichoderma: I. Production of non-volatile antibiotics. Transactions of the British Mycological Society.1971;57(1):25-IN3.

31. Kumar N, Kumar A, Sugha SK. Evaluation of bio-agents and plant extracts against alternaria blight of rapeseed-mustard. Plant Disease Research. 2006;35021 (1):48-50.

32. Robinson PM, Park D. Volatile inhibitors of spore germination produced by fungi. Transactions of the British Mycological Society.1966;49(4): 639-649.

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