

1
2 **Integrated effect of *Trichoderma harzianum***
3 **with selected botanicals extracts on *Alternaria***
4 **leaf spot of Broccoli (*Brassica oleracea* var.**
5 ***italica*) caused by *Alternaria brassicae* (Berk.)**
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17 **ABSTRACT**
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Broccoli (*Brassica 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 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 *Alternaria brassicae*. *In vitro* condition, five botanical extracts were taken at the concentration 10% and *Trichoderma harzianum* @10% and Mancozeb @0.2% (treated check). *In vivo* condition, eight treatments were taken and all seedlings are treated with *Trichoderma harzianum* @10%, Mancozeb @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 @10% were observed effective in the per cent disease inhibition (%) (79.77) followed by Eucalyptus leaf extract @10% (75.69). Among the treatments, under *In vivo* condition, T₃- *Trichoderma 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 (9.78cm, 23.84cm 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- *Trichoderma harzianum* (S.T.) and Neem leaf extract (F.S.) when compared to treated T₇- mancozeb (treated check) and T₀- Control (untreated check).

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20 **Keywords:** *Alternaria* leaf spot, *Alternaria brassicae*, botanicals plant extracts,
21 broccoli, *Trichoderma harzianum*.
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26 **1. INTRODUCTION**

27 Broccoli (*Brassica oleracea* var. *italica*) is an important cole crop vegetable, belong to
28 Brassicaceae family originated from Italy about more than 2000 years ago (**Buck,1956**).
29 Broccoli is native to the eastern mediterranean and asia minor, broccoli was cultivated in Italy
30 in ancient roman times. It was introduced to England in the 18th century and became popular
31 in the United States in the 20th century (**Moreno et al., 2007**). The common english name
32 broccoli' is derived from the Italian word 'broccolo', meaning 'the flowering crest of a cabbage'.
33 Fresh broccoli is dark green in colour with firm stalks and compact bud clusters. As a
34 vegetable, it is grown for its edible flower buds and stalk (**Das et al., 2021**).

35

36 Broccoli is a biennial plant belonging to the Brassicaceae family that is eaten as a vegetable
37 throughout the world. The edible plant parts are the stalk and large flowering head. Broccoli is
38 a rich source of vitamin C and vitamin K. Broccoli became one of the favourite foods due to its
39 high nutrient and fiber content. Broccoli also contains numerous phytochemicals, such as
40 polyphenols, namely kaempferol, quercetin glucosides, isorhamnetin, glucosinolates, and
41 their derivatives. These are responsible for its antioxidant and anticancer properties and other
42 health benefits. (**Nagraj et al., 2020**).

43

44 Broccoli is considered a cool season crop, which has now been distributed to both tropical and
45 subtropical areas (**Shivakumar et al., 2022**). This crop can be cultivated in various parts of
46 India during the winter season, especially in regions where rainfall is not excessive. Areas with
47 moderate rainfall and cooler temperatures are generally suitable for broccoli farming. Being a
48 cool season crop, it requires 15-20° C temperature for head creation. Temperature above 25°
49 C isn't favourable for its development and can cause slackening and darting of heads (**Kumar
50 et al., 2021**).

51

52 The global production of broccoli and cauliflower was 25,531,274 tons from an area of
53 1,357,186 ha, in which Europe ranks third (after China and India) and contributes 9.5 and
54 10.5% in production and area, respectively (**Siomos et al., 2022**). This vegetable, closely
55 resembling cauliflower but usually green in color, introduced in India many years after cabbage
56 and cauliflower and has gained popularity in short span of time. Now, India stands at second
57 position for cauliflower and broccoli production with an annual production of 6.7 million tonnes
58 (**Tejaswini et al.,2018**). It is mostly cultivated in hilly areas of Himachal Pradesh, Uttar
59 Pradesh, Jammu and Kashmir, Nilgiri Hills and Northern plains of India (**Meena et al., 2020**).

60

61 There are many diseases and pests collectively that lower the yield and quality of broccoli
62 crops. Broccoli is infected by various types of pathogens including bacteria, fungi, and viruses,
63 which lead to reduction of yield and quality (**Zhang et al., 2022**). Among all the diseases
64 *Alternaria* leaf spot disease caused by *Alternaria* sp. is the most important major disease which
65 resulted significant decline in the yield of all cultivars (**Lafi et al., 2022**).

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67 Among the different diseases caused by the genus *Alternaria*, *Alternaria* leaf spot disease
68 is most dominant one that causes average yield loss in the range of 32-57% (**Singh et al.,
69 2015**). During 2017 and 2018, severe symptoms of dark spots on leaves were observed on
70 broccoli plants (*Brassica oleracea* L. var. *italica*) cultivar with 45 to 37% disease incidence and
71 70% yield loss (**De Britto et al., 2020**).

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73 For the management of this disease fungicides have been recommended to manage the
74 disease, but present-day farmer perceptions and environmental hazards are compelling to
75 search for alternative eco-friendly disease management strategies (**Sakhare et al., 2021**) So
76 this situation compels to focus on disease management by utilizing biological agents, plant
77 extracts and fungicides in lowest concentration. Application of biological agents and extract is

78 eco-friendly and a sustainable approach apart from being a promising alternative to fungicide
 79 application. In the present study among the plant extracts, *Lantana camara* was found to be
 80 effective in managing the *Alternaria* blight infection in the field (71.92% reduction in disease
 81 severity and 68.18% increase in yield) in comparison to bio-agent (*Trichoderma viride*) 36.27%
 82 reduction in disease severity respectively. (Ahmad and Ashraf, 2016).

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84 **2. Materials and Methods**

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

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90 **2.1 Isolation and identification of pathogen**

91 The leaves were collected from infected plants bearing characteristic symptoms of *alternaria*
 92 leaf spot of broccoli. The leaves were thoroughly washed under running tap water. The
 93 symptoms on leaves after mounting on slide were examined under microscope to confirm the
 94 presence of *Alternaria* sp. The infected leaf parts along with the healthy portion was cut into
 95 small pieces under aseptic conditions and surface sterilized with 1% sodium hypo chlorite
 96 (NaOCl) solution for 1 minute and washed three times with sterile distilled water to remove
 97 any traces of sodium hypo chlorite (NaOCl) adhered with leaf bits. Then they were placed on
 98 filter paper so that extra water gets absorbed. After that five leaf bits were transferred on PDA
 99 media (Tuite, 1969) contained in sterilized petri plates with the help of forceps. To avoid
 100 bacterial contamination streptomycin @ 100 ppm, was added in the medium at lukewarm
 101 stage before pouring PDA into Petri plates. Then Petri plates were wrapped and incubated at
 102 $27\pm 2^{\circ}\text{C}$ in BOD, after 3 days mycelia growth was observed around leaf bits. With the help of
 103 cork borer a portion from the periphery having single hyphal tip from this colony growth was
 104 separated and transferred to other petri plates having medium to get pure culture and
 105 identification of the pathogen was recorded by observing the morphological features of colony,
 106 spore characteristics and referring the relevant literature (Barnett and Hunter, 1972).

107 **2.2 Evaluation of botanical extracts and *Trichoderma harzianum* against** 108 ***Alternaria* leaf spot of broccoli**

109 **2.2.1 *In vivo* evaluation**

110 Botanical extracts were prepared using method of standard procedure given by Mahapatra
 111 and Das (2013). Matured leaves and other botanicals were collected and sterilized with distilled
 112 water, the leaves were homogenized in a pestle and mortar, sterilized distilled water. Aqueous
 113 extract of this botanical (1% w/v) were prepared by mixing 100g fresh leaves / botanicals of
 114 plant with 100 ml of sterile distilled water and crushing in warring blender. The extract was
 115 filtered through four layers of moisture muslin cloth. The filtrate thus obtained was considered
 116 as stock solution. The phyto-extracts were sprayed @10% prepared from standard solution,
 117 Mancozeb at a ratio 0.2%, fungal bio-agent *Trichoderma harzianum* were also sprayed @10%
 118 after the appearance of the first symptoms in the field at thirty days after transplanting (DAT),
 119 the foliar spray of botanicals, Mancozeb and *Trichoderma harzianum* was applied three times
 120 at fifteen days interval. Unsprayed plots were kept as control.

121 **2.2.2 Disease Intensity**

122 The intensity of disease was visually assessed in all the plots at weekly interval from first
 123 appearance of disease for each treatment. Disease intensity was calculated using the formula
 124 given by Wheeler (1969).

$$\text{Disease Intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total no of ratings} \times \text{Maximum disease grade}} \times 100$$

125 **2.2.3 *In vitro* evaluation**

126 **Culture of *Trichoderma harzianum***

127 One gm of product was taken and mixed in nine ml of sterilized distilled water in a clean and
 128 sterilize test tube to make 10^{-1} dilution (1:10) shaken well and one ml of the suspension of nine
 129 ml of sterilize water was taken in a tube to make 10^{-2} dilution (1:100). Similarly, four more
 130 serial dilution in the same way was done to get 10^{-6} dilution and transferred one ml of this
 131 suspension to sterile petri plate containing 15-20ml of sterilized melted and cooled PDA media
 132 the plates were rotated gently and allowed to solidify. The petri plates were incubated in BOD
 133 incubator at $25^{\circ}\text{C} + 2^{\circ}\text{C}$ for five to seven days. The development of typical colony of
 134 *Trichoderma harzianum* was observed.

135 **Preparation of plant extracts**

136 The botanicals used for the experiment are Neem, Black night shade, Eucalyptus, Datura,
 137 Lantana. The botanical part used for the treatment were Neem leaf extract 10%, Black night
 138 shade leaf extract 10%, Eucalyptus leaf extract 10%, Datura leaf extract 10%, Lantana leaf
 139 extract 10%. Aqueous plant extracts were obtained as per the method described by **Bhatti,**
 140 **(1988)**. A 100-gram sample of each fresh leaves were gently wash under running tap water
 141 and again wash it in sterile distilled water. Then each sample was grind separately by using
 142 sterile pestle and mortal with 100 ml sterile distilled water. The extract of each sample
 143 thus obtained was filtered separately through a sterilized double layered muslin cloth to
 144 remove the bits of plant material is filtrate. Then extract was again filtered through a filter paper
 145 (Whatman No.1). This formed the standard plant extracts solution (100%). All the glassware
 146 used in the study were sterilized before use. All the plant extracts were tested at 10 per cent
 147 concentration against the test pathogen using PDA as a basal medium. To obtain 10 per cent
 148 concentration of plant extracts, 100 ml of lukewarm PDA was mixed with 10 ml of standard
 149 plant extracts in 250 ml conical flask, separately and then it was stirred well to obtain
 150 homogenized mixture.

151 **2.2.4 Dual Culture Technique**

152 Twenty ml of sterilized and cooled potato dextrose agar medium was poured into sterilized
 153 petri plates. Fungal antagonists were evaluated by inoculating a pathogen at one side of the
 154 petri plate and the antagonist at exactly opposite side of the same plate by leaving a space of
 155 4 cm. After required period of incubation i.e., when the growth in control plate records 90 mm
 156 in diameter, the radial growth of the pathogens was measured (**Vincent, 1947**).

157
 158 Percentage inhibition of mycelia growth of test pathogen was calculated using the formula:

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$$I = \frac{C - T}{C} \times 100$$

160 Where,

161 I = Percent reduction in growth of test pathogen

162 C = Radial growth (mm) in control

163 T = Radial growth (mm) in treatment

164 **2.2.5 Poisoned food technique**

165 Five mm diameter of culture disc of *Alternaria brassicae* was be kept at the center of each petri
 166 plate containing the botanicals of required concentration dissolved in PDA. Three replications
 167 were maintained. The plates were incubated at $27 \pm 2^{\circ}\text{C}$ for seven days and colony diameter
 168 was recorded (**Vincent, 1947**).

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

$$I = \frac{C - T}{C} \times 100$$

171 Where,

172 I = Percent inhibition

173 C = Growth (mm) of test fungus in untreated control plate

174 T = Growth (mm) of test fungus in treated plate

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176 3. RESULTS AND DISCUSSION

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178 3.1 Effect of plant extracts on *Alternaria brassicae* by poison food technique

179 Radial growth (mm) of *Alternaria brassicae*

180 The data presented in table 1, depicted reveal significant reduction in T₃- Neem leaf extract
 181 (18.20mm) followed by T₂ – Eucalyptus leaf extract (21.87mm), T₅ – Black night shade leaf
 182 extract (23.83mm), T₄ – Lantana leaf extract (26.00mm), T₁- Datura leaf extract
 183 (26.98mm) as compared to mancozeb (treated check)- (2.50mm) and untreated check
 184 control T₀ - (90mm).

185 Percent inhibition of mycelia growth

186 The data present presented in table 1 and figure 1 reveal that there was significant increase
 187 in T₃- Neem leaf extract @10% (79.77%) followed by T₂ – Eucalyptus leaf extract @10%
 188 (75.7%), T₅ – Black night shade leaf extract @10% (73.52%), T₄ – Lantana leaf extract @10%
 189 (71.11%), T₁- Datura leaf extract @10% (70.02%) as compared to T₇-mancozeb (treated
 190 check) (97.21%) and untreated check control T₀ - (0%). The probable reason for such findings
 191 may be due to the fact that neem leaf contains active compounds such as azadirachtin as well
 192 as salanin, nimbim. Constituents such as nimbim, nimbidin, limonoids which disrupts the cell
 193 of the pathogen and restricts the performance of some enzymes that are important to proliferate
 194 fungi in nature and leads to their death. The current findings are consistent with the research
 195 conducted by **Vijaykumar et al. (2022)** and **Kumar et al. (2007)** who tested the effectiveness
 196 of different phytoextracts against *Alternaria brassicae* and found that the antifungal activity
 197 and potentiality of nimbicidin and neem leaf extract have been proved successfully against
 198 *Alternaria brassicae*.

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202 3.2 Effect of treatments on disease intensity of *Alternaria* leaf spot of broccoli 203 caused by *Alternaria brassicae*

204 The data presented in the table 2 reveal that the disease intensity (%) on broccoli significantly
 205 reduced by T₃ – *Trichoderma harzianum* + Neem leaf extract @10% (23.28%) followed by T₆-
 206 (*Trichoderma harzianum* @10% (26.31%), T₅ – (*Trichoderma harzianum* + Black night shade
 207 leaf extract @10% (30.26%), T₄- (*Trichoderma harzianum* + Lantana leaf extract @ 10%
 208 (32.56%), T₂ – *Trichoderma harzianum* + Eucalyptus leaf extract @10% (36.83%), T₁-
 209 (*Trichoderma harzianum* + Datura leaf extract @10% (37.28%) as compared to the T₇ –
 210 Mancozeb @ 0.2% (treated check) (18.96%) as well as (T₀) – control (untreated check)
 211 (40.64%). Similar findings have been reported by **Anshika and Zacharia (2023)** and **Ravella**
 212 **et al. (2023)** The probable reason for this result may be due to the neem having anti- microbial
 213 activity. Neem has reported to exhibit antifungal, antibacterial and insecticidal properties. The
 214 ethanolic extract of neem leaves stops the growth of fungi. *Trichoderma harzianum* has shown
 215 significant results on disease intensity and yield attributes, whereas yield has been
 216 significantly increased and decreased disease intensity which may be due to the mycoparasite

217 nature and ability to generate volatile and non-volatile compounds against pathogen with great
218 anti- microbial activity.

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220 **3.3 Effect of treatments on plant height (cm) of broccoli**

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222 The data presented in the table 3 reveal that the plant height (cm) on broccoli significantly
223 increased plant height in T₃ –(*Trichoderma harzianum* + Neem leaf extract @10% (36.66
224 cm) followed by T₆- (*Trichoderma harzianum* @10% (33.70 cm), T₅ – (*Trichoderma*
225 *harzianum* + Black night shade leaf extract @10% (31.10 cm), T₂- (*Trichoderma harzianum*
226 + Eucalyptus leaf extract @ 10% (29.93 cm), T₄ –(*Trichoderma harzianum* + Lantana leaf
227 extract @10% (28.41 cm), T₁- (*Trichoderma harzianum* + Datura leaf extract @10% (27.78
228 cm) as compared to the T₇ – Mancozeb @ 0.2% (treated check)(39.47 cm) as well as (T₀)
229 – control (untreated check) (23.01 cm). The probable reason for this result may be due to
230 neem aqueous extract showed a promotive effect on shoot lengths, branches and leaves
231 numbers. The bio-efficacy of neem extract over pathogens can be attributed to the fact that
232 neem has active compounds such as azadirachtin, nimbin, nimbidin, nimbinene and
233 azadirone which are antifungal, anti-bacterial and anti-insecticidal in nature **Nahak et al.**
234 **(2015)**. *Trichoderma* strains colonise plant roots, establishing chemical communication and
235 systemically altering the expression of numerous plant genes that alter plant physiology.
236 Application of *Trichoderma harzianum* significantly increased curd circumference length, the
237 diameter of the stem **Rosa et al. (2019)**.

238 **3.4 Effect of treatments on number of leaves per plant of broccoli**

239 The data presented in table 4 reveal that number of leaves significantly increased in T₃ –
240 (*Trichoderma harzianum* + Neem leaf extract @10% (28.00) followed by T₆- (*Trichoderma*
241 *harzianum* @10% (25.20), T₅ – (*Trichoderma harzianum* + Black night shade leaf extract
242 @10% (23.40), T₂- (*Trichoderma harzianum* + Eucalyptus leaf extract @ 10% (21.13), T₄
243 –(*Trichoderma harzianum* + Datura leaf extract @10% (20.66), T₁- (*Trichoderma*
244 *harzianum* + Lantana leaf extract @10% (20.40) as compared to the T₇ – Mancozeb @
245 0.2% (treated check) (31.53) as well as (T₀) control (untreated check) (16.00). The probable
246 reason for this result may be due to neem aqueous extract showed a promotive effect on
247 shoot lengths, branches and leaves numbers. The bio-efficacy of neem extract over
248 pathogens can be attributed to the fact that neem has active compounds such as
249 azadirachtin, nimbin, nimbidin, nimbinene and azadirone which are antifungal, antibacterial
250 and anti-insecticidal in nature **Nahak et al. (2015)**. *Trichoderma* strains colonise plant roots,
251 establishing chemical communication and systemically altering the expression of numerous
252 plant genes that alter plant physiology. Application of *Trichoderma harzianum* significantly
253 increased curd circumference length, the diameter of the stem, and the leaf greenness index
254 value **Rosa et al. (2019)**.

255 **3.5 Effect of treatments on head weight (kg) of broccoli**

256 The data presented in table 5 and figure 3 reveal that head weight (kg) of broccoli
257 significantly increased in T₃ – *Trichoderma harzianum* + Neem leaf extract @10% (0.38 kg)
258 followed by T₆- *Trichoderma harzianum* @10% (0.35 kg), T₅ – *Trichoderma harzianum* +
259 Black night shade leaf extract @10% (0.304 kg), T₄- *Trichoderma harzianum* + Lantana leaf
260 extract @ 10% (0.301 kg), T₂ – (*Trichoderma harzianum* + Eucalyptus @10% (0.21), T₁-
261 *Trichoderma harzianum* + Datura leaf extract @10% (0.20kg) as compared to the T₇ –
262 Mancozeb @ 0.2% (treated check) (0.40 kg) as well as (T₀) – control(untreated check)
263 (0.16 kg). The probable reason for such findings may be that the *Trichoderma* and plant
264 interaction produce the secondary metabolites such as auxin-like compounds or auxin-

265 inducing substances that resulted in improved growth, head diameter and yield **Tanwar et**
 266 **al. (2013)**. Neem extract is composed of antimicrobial ingredients such as alkaloids,
 267 glycosides, flavonoids, and saponins, which are common antibiotics found in plants. At
 268 concentrations of 0.1 and 0.4 gmL⁻¹, neem extract inhibited the growth of soil
 269 microorganisms **Sarawaneeyaruk et al. (2015)**.

270 3.6 Effect of treatments on yield (t/ha) of broccoli

271 The data presented in table 6 and figure 4 revealed that yield of broccoli significantly
 272 increased head weight T3 – *Trichoderma harzianum* + Neem leaf extract @10% (9.68
 273 t/ha) followed by T6- *Trichoderma harzianum* @10% (7.92 t/ha), T5 – (*Trichoderma*
 274 *harzianum* + Black night shade leaf extract @10% (6.81 t/ha), T4- (*Trichoderma harzianum*
 275 + Lantana leaf extract @ 10% (6.50 t/ha), T4 –(*Trichoderma harzianum* + Eucalyptus leaf
 276 extract @10% (6.19 t/ha), T1- (*Trichoderma harzianum* + Datura leaf extract @10% (5.68
 277 t/ha) as compared to the T7 – Mancozeb @ 0.2% (treated check) (11.55 t/ha) as well as
 278 (T0) – control (untreated check) (3.85 t/ha). The probable reason for this result may be due
 279 to the production of secondary metabolites such as antibiotics, isocyanide, acids and cell wall
 280 degrading enzymes which are implicated in the growth in the growth inhibition of many
 281 phytopathogenic fungi **Supriya et al. (2022)**. *Trichoderma viride* has shown significant
 282 results on yield attributes, the yield has been significantly increased which may be attributed
 283 to the mycoparasitic nature and ability to generate volatile and non-volatile compounds
 284 against pathogen with great anti-microbial activity (**Ravella et al., 2023**).

285 **Table 1. Effect of selected plant extracts on radial growth (mm) of *Alternaria***
 286 ***brassiccae***

Sr. No.	Treatments	Concentration	Mean Colony diameter (mm)	Percent inhibition
T0	Control (untreated check)	-	90.00	0.00
T1	Datura leaf extract	10%	26.98 ^a	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%	26.00 ^a	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

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Figure 1 :- Efficacy of plant extract on percent mycelial inhibition of *Alternaria brassicae* by poisoned food technique

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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	<i>Trichoderma harzianum</i> 10% (S.T.) + Datura leaf extract 10%(F.S.)	23.40	29.88	37.28
T ₂	<i>Trichoderma harzianum</i> 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	22.50	29.55	36.83
T ₃	<i>Trichoderma harzianum</i> 10% (S.T.) + Neem leaf extract 10%(F.S.)	16.59	19.85	23.28
T ₄	<i>Trichoderma harzianum</i> 10% (S.T.) + Lantana leaf extract 10%(F.S.)	21.34	27.51	32.56
T ₅	<i>Trichoderma harzianum</i> 10% (S.T.) + Black night shade leaf extract 10%(F.S.)	20.28	25.64	30.26
T ₆	<i>Trichoderma harzianum</i> 10% (S.T.) + <i>Trichoderma harzianum</i> 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

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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	<i>Trichoderma harzianum</i> 10% (S.T.) + Datura leaf extract 10%(F.S.)	7.46	17.06	27.78
T ₂	<i>Trichoderma harzianum</i> 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	8.96	19.16	29.93
T ₃	<i>Trichoderma harzianum</i> 10% (S.T.) + Neem leaf extract 10%(F.S.)	9.78	23.84	36.66
T ₄	<i>Trichoderma harzianum</i> 10% (S.T.) + Lantana leaf extract 10%(F.S.)	8.21	18.28	28.41

T ₅	<i>Trichoderma harzianum</i> 10% (S.T.) + Black night shade leaf extract 10%(F.S.)	9.64	20.12	31.10
T ₆	<i>Trichoderma harzianum</i> 10% (S.T.) + <i>Trichoderma harzianum</i> 10%(F.S.)	10.43	21.37	33.70
T ₇	Mancozeb (treated check) 0.2% (S.T. +F.S.)	12.72	26.43	39.47
S.Em. (+)		0.17	0.40	0.45
C.D (5%)		0.53	1.23	1.31

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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	<i>Trichoderma harzianum</i> 10% (S.T.) + Datura leaf extract 10%(F.S.)	8.16	12.40	20.66
T ₂	<i>Trichoderma harzianum</i> 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	8.93	13.40	21.13
T ₃	<i>Trichoderma harzianum</i> 10% (S.T.) + Neem leaf extract 10%(F.S.)	9.36	17.26	28.00
T ₄	<i>Trichoderma harzianum</i> 10% (S.T.) + Lantana leaf extract 10%(F.S.)	8.46	12.93	20.40
T ₅	<i>Trichoderma harzianum</i> 10% (S.T.) + Black night shade leaf extract 10%(F.S.)	8.86	14.16	23.40
T ₆	<i>Trichoderma harzianum</i> 10% (S.T.) + <i>Trichoderma harzianum</i> 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

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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	<i>Trichoderma harzianum</i> 10% (S.T.) + Datura leaf extract 10%(F.S.)	0.20
T ₂	<i>Trichoderma harzianum</i> 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	0.21
T ₃	<i>Trichoderma harzianum</i> 10% (S.T.) + Neem leaf extract 10%(F.S.)	0.38
T ₄	<i>Trichoderma harzianum</i> 10% (S.T.) + Lantana leaf extract 10%(F.S.)	0.301
T ₅	<i>Trichoderma harzianum</i> 10% (S.T.) + Black night shade leaf extract 10%(F.S.)	0.304
T ₆	<i>Trichoderma harzianum</i> 10% (S.T.) + <i>Trichoderma harzianum</i> 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

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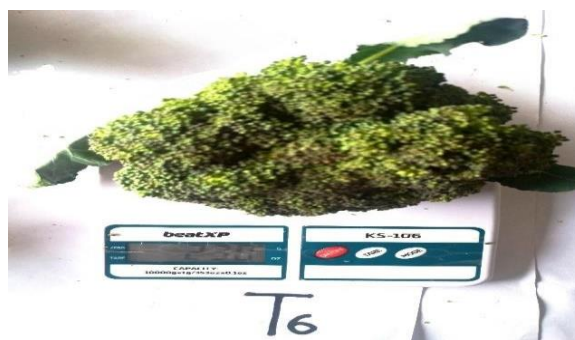


Figure 2:- Weighing of broccoli

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

Sr. No.	Treatments	Yield (t/ha)
T ₀	Control (untreated check)	3.85
T ₁	<i>Trichoderma harzianum</i> 10% (S.T.) + Datura leaf extract 10%(F.S.)	5.68
T ₂	<i>Trichoderma harzianum</i> 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	6.19
T ₃	<i>Trichoderma harzianum</i> 10% (S.T.) + Neem leaf extract 10%(F.S.)	9.73
T ₄	<i>Trichoderma harzianum</i> 10% (S.T.) + Lantana leaf extract 10%(F.S.)	6.50
T ₅	<i>Trichoderma harzianum</i> 10% (S.T.) + Black night shade leaf extract 10%(F.S.)	6.81
T ₆	<i>Trichoderma harzianum</i> 10% (S.T.) + <i>Trichoderma harzianum</i> 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

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Figure 3:- Yield of broccoli

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4. CONCLUSION

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

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