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# Integrated effect of *Trichoderma harzianum* with selected botanical extracts on *Alternaria* leaf spot of broccoli (*Brassica oleracea* var. *italica*) caused by *Alternaria brassicae* (Berk.) Sacc.

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## ABSTRACT

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 *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 T<sub>0</sub> – control (untreated check) and (treated check). *In vitro* condition, among the treatments Neem leaf extract at 10% were observed effective in the percent disease inhibition (%) (79.77) followed by Eucalyptus leaf extract at 10% (75.69). Among the treatments, under *In vivo* condition, T<sub>3</sub>- *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 36.66 cm) 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 T<sub>3</sub>- *T. harzianum* (S.T.) and Neem leaf extract (F.S.) when compared to treated T<sub>7</sub>- mancozeb (treated check) and T<sub>0</sub>- Control (untreated check).

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**Keywords:** *Alternaria* leaf spot, *Alternaria brassicae*, botanicals plant extracts, 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 times. It was introduced to England in the 18<sup>th</sup> century and became popular  
 31 in the United States in the 20<sup>th</sup> 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**).

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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**).

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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 is not favourable for its development and can cause slacking and darting of heads (**Kumar  
 50 et al., 2021**).

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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**).

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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 (**Zhan 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 in 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 the 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 spot 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 extracts is

eco-friendly and a sustainable approach apart from being a promising alternative of 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. (Ahmad and Ashraf, 2016).

## 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, Technology 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 slides 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 hypochlorite (NaOCl) solution for 1 minute and washed three times with sterile distilled water to remove any traces of sodium hypochlorite (NaOCl) adhered with leaf bits. Then they were placed on filter papers so that extra water gets absorbed. After that five leaf bits were transferred on PDA media (Tuite, 1969) contained in sterilized petriplates with the help of forceps. To avoid bacterial contamination streptomycin @ 100 ppm, was added in the medium at lukewarm stage before pouring PDA into Petriplates. Then Petriplates 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 petriplates 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 (Barnett and Hunter, 1972).

### 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 (2013). 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 100ml of sterile distilled water and crushing in a 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 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 (1969).

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

#### 2.2.3 In vitro evaluation

### 126 **Culture of *T. harzianum***

127 One g of product was taken and mixed in nine ml of sterilized distilled water in a clean and  
 128 sterilized test tube to make  $10^{-1}$  dilution (1:10) shaken well and one ml of the suspension of nine  
 129 ml of sterilized water was taken in a tube to make  $10^{-2}$  dilution (1:100). Similarly, four more  
 130 serial dilutions in the same way were done to get  $10^{-6}$  dilution and transferred one ml of this  
 131 suspension to sterile petri plates containing 15-20 ml 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  $25 \pm 2^\circ\text{C}$  for five to seven days. The development of typical colonies of  
 134 *T. harzianum* was observed.

### 135 **Preparation of plant extracts**

136 The botanicals used for the experiment were Neem, Black nightshade, Eucalyptus, Datura,  
 137 Lantana. The botanical parts 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 leaf was gently washed under running tap water  
 141 and again washed in sterile distilled water. The each sample was ground separately by using  
 142 sterile pestle and mortar with 100 ml sterile distilled water. The extract of each sample  
 143 thus obtained was filtered separately through sterilized double layered muslin cloth to  
 144 remove the bits of plant material as filtrate. The extract was again filtered through filter paper  
 145 (Whatman No. 1). This formed the standard plant extract solution (100%). All the glassware  
 146 used in the study were sterilized before use. All the plant extracts were tested at 10 percent  
 147 concentration against the test pathogen using PDA as basal medium. To obtain 10 percent  
 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 pathogen was measured (**Vincent, 1947**).

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

$$159 \quad I = \frac{C - T}{C} \times 100$$

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 cultured disc of *A. brassicae* was 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 Percentage 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 extract on *A. brassicae* by poison food technique

##### 179 Radial growth (mm) of *A. brassicae*

180 The data presented in table 1, depicted reveals significant reduction in T<sub>3</sub>-Neem leaf extract  
 181 (18.20mm) followed by T<sub>2</sub>-Eucalyptus leaf extract (21.87mm), T<sub>5</sub>-Black nightshade leaf  
 182 extract (23.83mm), T<sub>4</sub>-Lantana leaf extract (26.00mm), T<sub>1</sub>-Datura leaf extract  
 183 (26.98mm) as compared to mancozeb (treated check) (2.50mm) and untreated check  
 184 control T<sub>0</sub> (90mm).

##### 185 Percent inhibition of mycelial growth

186 The data presented in table 1 and figure 1 reveal that there was significant increase  
 187 in T<sub>3</sub>-Neem leaf extract at 10% (79.77%) followed by T<sub>2</sub>-Eucalyptus leaf extract at 10%  
 188 (75.7%), T<sub>5</sub>-Black nightshade leaf extract at 10% (73.52%), T<sub>4</sub>-Lantana leaf extract at 10%  
 189 (71.11%), T<sub>1</sub>-Datura leaf extract at 10% (70.02%) as compared to T<sub>7</sub>-mancozeb (treated  
 190 check) (97.21%) and untreated check control T<sub>0</sub> (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 salannin, nimbim. Constituents such as nimbim, nimbidin, limonoids which disrupt the cell  
 193 of the pathogen and restrict the performance of some enzymes that are important to proliferate  
 194 fungi in nature and lead 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 *A. brassicae* and found that the antifungal activity  
 197 and potentiality of nimbicidin and **Neem** leaf extract have been proved successfully against  
 198 the causal fungus..

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

204 The data presented in the Table (2) reveal that the disease intensity (%) on broccoli is significantly  
 205 reduced by T<sub>3</sub>-*T. harzianum*+Neem leaf extract at 10% (23.28%) followed by T<sub>6</sub>-  
 206 (*T. harzianum* at 10% (26.31%), T<sub>5</sub>-(*T. harzianum*+Black nightshade  
 207 leaf extract at 10% (30.26%), T<sub>4</sub>-(*T. harzianum*+Lantana leaf extract at 10%  
 208 (32.56%), T<sub>2</sub>-*T. harzianum*+Eucalyptus leaf extract at 10% (36.83%), T<sub>1</sub>-  
 209 (*T. harzianum*+Datura leaf extract at 10% (37.28%) as compared to the T<sub>7</sub>-  
 210 mancozeb at 0.2% (treated check) (18.96%) as well as (T<sub>0</sub>)-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. *T. 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 plantheight (cm) of broccoli

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222 The data presented in the Table (3) reveal that the plantheight (cm) on broccoli significantly  
223 increased plantheight in T<sub>3</sub>– (*T.harzianum*+Neem leaf extract at 10% (36.66  
224 cm) followed by T<sub>6</sub>– (*T.harzianum* at 10% (33.70 cm), T<sub>5</sub>– (*T.  
225 harzianum*+Black nightshade leaf extract @ 10% (31.10 cm), T<sub>2</sub>– (*T.harzianum*  
226 +Eucalyptus leaf extract at 10% (29.93 cm), T<sub>4</sub>– (*T.harzianum*+Lantana leaf  
227 extract at 10% (28.41 cm), T<sub>1</sub>– (*T.harzianum*+Datura leaf extract at 10% (27.78  
228 cm) as compared to the T<sub>7</sub>– Mancozeb at 0.2% (treated check) (39.47 cm) as well as (T<sub>0</sub>)  
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 **Nahaketal.  
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 *T.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 T<sub>3</sub>–  
240 (*T.harzianum*+Neem leaf extract at 10% (28.00) followed by T<sub>6</sub>– (*T.  
241 harzianum* at 10% (25.20), T<sub>5</sub>– (*T.harzianum*+Black nightshade leaf extract  
242 at 10% (23.40), T<sub>2</sub>– (*T.harzianum*+Eucalyptus leaf extract at 10% (21.13), T<sub>4</sub>  
243 – (*T.harzianum*+Datura leaf extract at 10% (20.66), T<sub>1</sub>– (*T.harzianum*+Lantana  
244 leaf extract at 10% (20.40) as compared to the T<sub>7</sub>– Mancozeb at  
245 0.2% (treated check) (31.53) as well as (T<sub>0</sub>) 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 **Nahaketal. (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 *T.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 the head weight (kg) of broccoli  
257 significantly increased in T<sub>3</sub>– *T.harzianum*+Neem leaf extract at 10% (0.38 kg)  
258 followed by T<sub>6</sub>– *T.harzianum* at 10% (0.35 kg), T<sub>5</sub>– *T.harzianum*+  
259 Black nightshade leaf extract at 10% (0.304 kg), T<sub>4</sub>– *T.harzianum*+Lantana leaf  
260 extract at 10% (0.301 kg), T<sub>2</sub>– (*T.harzianum*+Eucalyptus at 10% (0.21), T<sub>1</sub>–  
261 *T.harzianum*+Datura leaf extract at 10% (0.20 kg) as compared to the T<sub>7</sub>–  
262 Mancozeb at 0.2% (treated check) (0.40 kg) as well as (T<sub>0</sub>) –control (untreated check)  
263 (0.16 kg). The probable reason for such findings may be that the *Trichoderma* and plant  
264 interaction produce these secondary metabolites such as auxin-like compounds or auxin-

265 inducing substances that resulted in improved growth, head diameter and yield **Tanwaret**  
 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 g mL<sup>-1</sup>, **Neem** extract inhibited the growth of soil  
 269 microorganisms **Sarwaneeyaruk et al. (2015)**.

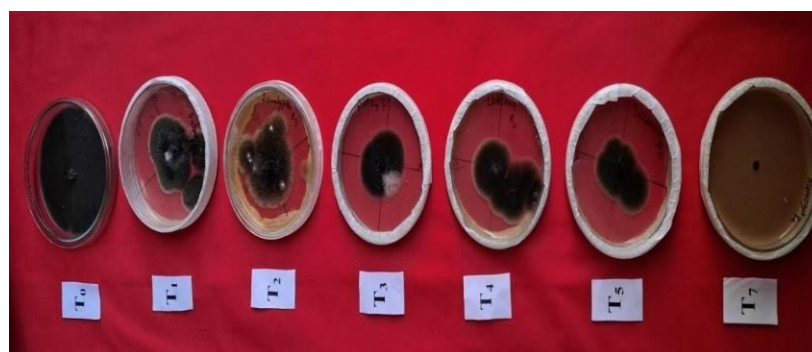
270 **3.6 Effect of treatments on yield (t/ha) of broccoli**

271 The data presented in **Table (6)** and **Figure (4)** revealed that yield of broccoli significantly  
 272 increased head weight T3- *T. harzianum*+Neem leaf extract at 10% (9.68  
 273 t ha<sup>-1</sup>) followed by T6- *T. harzianum* at 10% (7.92 t/ha), T5- (*T.*  
 274 *harzianum*+Black nightshade leaf extract at 10% (6.81 t ha<sup>-1</sup>), T4- (*T. harzianum*  
 275 +Lantana leaf extract at 10% (6.50 t ha<sup>-1</sup>), T4- (*T. harzianum*+Eucalyptus leaf  
 276 extract at 10% (6.19 t ha<sup>-1</sup>), T1- (*T. harzianum*+Datura leaf extract @ 10% (5.68  
 277 t ha<sup>-1</sup>) as compared to the T7- Mancozeb at 0.2% (treated check) (11.55 t ha<sup>-1</sup>) 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 inhibition of many  
 281 phytopathogenic fungi **Supriya et al. (2022)**. *T. viride* has shown significant  
 282 result 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 *A.***  
 286 ***brassicacae***

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 <sup>a</sup>	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 <sup>a</sup>	71.10
T5	Black nightshade leaf extract	10%	23.83	73.51
T6	Mancozeb	0.2%	2.50	97.21
<b>S.Em.(+)</b>			0.51	0.57
<b>C.D (5%)</b>			1.59	1.76

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**Figure1:-Efficacyofplantextractonpercentmycelial inhibitionof *A.brassicae*by poisoned food technique.**

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**Table2.Percentdiseaseintensity(%)at45,60and75DATasaffectedbytreatments**

Sr.No.	Treatments	45DAT	60DAT	75DAT
T <sub>0</sub>	Control(untreatedcheck)	25.98	33.83	40.64
T <sub>1</sub>	<i>T.harzianum</i> 10%(S.T.)+Datura leafextract10%(F.S.)	23.40	29.88	37.28
T <sub>2</sub>	<i>T.harzianum</i> 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	22.50	29.55	36.83
T <sub>3</sub>	<i>T.harzianum</i> 10%(S.T.)+Neem leaf extract 10%(F.S.)	16.59	19.85	23.28
T <sub>4</sub>	<i>T.harzianum</i> 10%(S.T.)+ Lantana leafextract10%(F.S.)	21.34	27.51	32.56
T <sub>5</sub>	<i>T.harzianum</i> 10%(S.T.)+Black night shade leaf extract 10%(F.S.)	20.28	25.64	30.26
T <sub>6</sub>	<i>T.harzianum</i> 10% (S.T.) + <i>T.harzianum</i> 10%(F.S.)	18.26	22.36	26.31
T <sub>7</sub>	Mancozeb(treatedcheck)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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**Table3.Effectoftreatmentsonplantheight(cm)ofbroccoli at30,60and90DAT**

Sr.No.	Treatments	30DAT	60DAT	90DAT
T <sub>0</sub>	Control(untreatedcheck)	6.92	15.40	23.01
T <sub>1</sub>	<i>Trichodermaharzianum</i> 10% (S.T.) + Datura leaf extract 10%(F.S.)	7.46	17.06	27.78
T <sub>2</sub>	<i>Trichoderma harzianum</i> 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	8.96	19.16	29.93
T <sub>3</sub>	<i>Trichodermaharzianum</i> 10%(S.T.)+Neem leaf extract 10%(F.S.)	9.78	23.84	36.66
T <sub>4</sub>	<i>Trichodermaharzianum</i> 10%(S.T.)+Lantana leaf extract 10%(F.S.)	8.21	18.28	28.41

T <sub>5</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Black nightshade leaf extract 10%(F.S.)	9.64	20.12	31.10
T <sub>6</sub>	<i>Trichoderma harzianum</i> 10% (S.T.) + <i>Trichoderma harzianum</i> 10%(F.S.)	10.43	21.37	33.70
T <sub>7</sub>	Mancozeb(treated check)0.2%(S.T.+F.S.)	12.72	26.43	39.47
<b>S.Em.(+)</b>		0.17	0.40	0.45
<b>C.D(5%)</b>		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 <sub>0</sub>	Control(untreated check)	7.73	10.46	16.00
T <sub>1</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Datura leaf extract 10%(F.S.)	8.16	12.40	20.66
T <sub>2</sub>	<i>Trichoderma harzianum</i> 10% (S.T.) + Eucalyptus leaf extract 10%(F.S.)	8.93	13.40	21.13
T <sub>3</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Neem leaf extract 10%(F.S.)	9.36	17.26	28.00
T <sub>4</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Lantana leaf extract 10%(F.S.)	8.46	12.93	20.40
T <sub>5</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Black night shade leaf extract 10%(F.S.)	8.86	14.16	23.40
T <sub>6</sub>	<i>Trichoderma harzianum</i> 10% (S.T.) + <i>Trichoderma harzianum</i> 10%(F.S.)	9.76	15.73	25.20
T <sub>7</sub>	Mancozeb(treated check)0.2%(S.T.+F.S.)	10.13	20.13	31.53
<b>S.Em.(+)</b>		0.11	0.21	0.21
<b>C.D(5%)</b>		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 <sub>0</sub>	Control(untreated check)	0.16
T <sub>1</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Datura leaf extract 10%(F.S.)	0.20
T <sub>2</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Eucalyptus leaf extract 10%(F.S.)	0.21
T <sub>3</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Neem leaf extract 10%(F.S.)	0.38
T <sub>4</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Lantana leaf extract 10%(F.S.)	0.301
T <sub>5</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+Black night shade leaf extract 10%(F.S.)	0.304
T <sub>6</sub>	<i>Trichoderma harzianum</i> 10%(S.T.)+ <i>Trichoderma harzianum</i> 10%(F.S.)	0.35
T <sub>7</sub>	Mancozeb(treated check)0.2%(S.T.+F.S.)	0.40
<b>S.Em.(+)</b>		0.05
<b>C.D(5%)</b>		0.15

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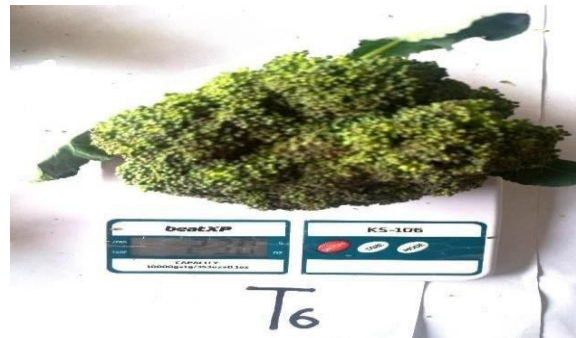


Figure2:-Weighingofbroccoli

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Table6.Effectoftreatmentsontotalyield(t/ha)ofbroccoli

Sr.No.	Treatments	Yield(t ha <sup>-1</sup> )
T <sub>0</sub>	Control(untreatedcheck)	3.85
T <sub>1</sub>	<i>Trichodermaharzianum</i> 10%(S.T.)+Daturaleafextract 10%(F.S.)	5.68
T <sub>2</sub>	<i>Trichodermaharzianum</i> 10%(S.T.)+Eucalyptusleafextract 10%(F.S.)	6.19
T <sub>3</sub>	<i>Trichodermaharzianum</i> 10%(S.T.)+Neemleafextract 10%(F.S.)	9.73
T <sub>4</sub>	<i>Trichodermaharzianum</i> 10%(S.T.)+Lantanaleafextract 10%(F.S.)	6.50
T <sub>5</sub>	<i>Trichodermaharzianum</i> 10%(S.T.)+Blacknightshadeleaf extract10%(F.S.)	6.81
T <sub>6</sub>	<i>Trichodermaharzianum</i> 10%(S.T.)+ <i>Trichodermaharzianum</i> 10%(F.S.)	7.35
T <sub>7</sub>	Mancozeb(treatedcheck)0.2%(S.T.+F.S.)	11.55
<b>S.Em.(+)</b>		0.12
<b>C.D(5%)</b>		0.37

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**Figure3:-Yieldofbroccoli**

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#### **4.CONCLUSION**

322 It can be concluded that *T.harzianum* and botanicals Neem leaf extract is  
323 significant in controlling *A.brassicae* in broccoli. This study contributes valuable  
324 insights into sustainable disease management practices by the use 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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330 **Please write references according to the journal's system, then check them with those  
on the manuscript.**

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