Original Research Article

Effectsofbio-agentsandselectedbotanicalsonAlternarialeafspotcausedby *Alternariaalternata*(Fr.)Keisslerinbroccoli(*Brassicaoleracea*var.*italica*L.)

ABSTRACT

A field experiment was conducted at the research plot of the Department of Plant Pathology, SHUATS, Prayagraj, Uttar Pradesh in *Rabi* season of 2023 to evaluate eight treatments *viz.*, T_1– (*Pseudomonas fluorescens* (S.T) @ 2%, T_2– *Trichoderma viride* (S.T) @ 2%, T_3– *Pseudomonas fluorescens* (S.T) @ 2% + *Ocimum sanctum* (F.S) @ 10 %, T_4- *Trichoderma viride* (S.T) @ 2 % + *Allium sativum* (F.S) @10%, T_5– *Trichoderma viride* (S.T) @ 2% + *Ocimum sanctum* (F.S) @ 10%,T_6 - *Pseudomonas fluorescens* (S.T) @ 2% + *Allium sativum* (F.S) @ 10 %, T_7 – Carbendazim @ 0.1% (F.S) (treated control) and control T_0(untreated control) with three replications in Randomized Block Design (RBD) for management of alternaria leaf spot of broccoli. Study on disease intensity of alternaria leaf spot of broccoli in field conditions revealed that the Minimum percent disease intensity (%) was recorded in T_4 –28.17% *Trichodermaviride* @ 2% (S.T) combined with *Allium sativum* @ 10% (F.S)as compared with the untreated control T_0 – 38.31 % and treated check T_7 – 24.42 % (Carbendazim @ 0.1% (F.S). PDI recorded in broccoli with maximum cost benefit ratio per treatment was recorded 1:1.915 in T^4 - *Trichodermaviride* @ 2% (S.T) combined with *Allium sativum* @ 10% (F.S) in broccoli.

Key words: *Botanical extracts, Foliar Spray, Soil Treatment, alternaria leaf spot, growth parameters, Ocimum sanctum, Allium sativum, broccoli and Percent Disease Intensity.*

INTRODUCTION

Broccoli(*Brassicaoleracea*var.*italica*L.)isanimportantandhighlyexoticandnutritiousvegetable.It is a member of the cruciferous family originated from Italy about more than 2000 years ago (Buck,1956).Itisalsoknownaswinter broccoliandheadingbrocco-li.Itisconsideredtobeoriginatedfromwild cabbage. *Brassica oleracea* var. *oleracea*, which is growing along the Mediterranean Sea.Broccoli has large flower curds arranged in a tree- like structure on branched sprouts growing from athick, edible stalk. Broccoli is a high-quality vege-table for fresh use and is one of the most popularfrozenvege-tables.Itisalsousedasavegetableinmanyothercountries,suchasSpain,Mexico,Italy,France andtheU-nitedStates(Patel*etal*.,2023).

It is a winter season crop and grown as biennial crop for seed production, but in tropical andsub-tropical areas it mainly grown as annual vegetable crop. Forbroccoliproduction, the ideal tem-perature required 25°C to 26°C during the day and 16°C to 17°C at night(Ferdinand*etal*., 2022).

Thedemand forbroccoliincreasesworldwide-by4percentannuallyduetoitsessentialhealthbenefits.Specifically,it hasbeenreportedtohaveabenefi-cialeffectagainstvarioustypesofcancer,such aslung,prostate and breast, since its high content of antioxidant compounds like carotene (1.9 mg caroteneper100g offreshbrocco-li).Nutritionally,itisrichinvitamin-A (2500I.U.),vitamin C (113mg),protein(3.6g),carbohydrates(5.9g)and mineralslikecalcium(103mg),iron(1.1mg), phos-phorous(78mg),potassium(382mg)andsodium(15mg)per100gofanedibleportion(USDA,2023).

TheleadingproducersofbroccoliareChinawith36.83 percent,Indiawith36.74 per-cent,theUnitedStates of America (US) with 3.56 per cent, Mexico with 2.84 per cent and Spain with 2.60 per centotherswith18 percent(FAO,2023).

The major broccoli diseases are damping off, club root of crucifers or finger and toe dis-ease,alternarialeafspot,blackrot,downymildewandwhiterust.Alternarialeafspotisoneofthefactorsresponsibleforeconomiclossesinbroccoli(Lafi*etal*., 2022).

Among all the diseases alternaria leaf spot caused by *Alternaria* sp. has become a problematicdi-sease due to the soil-borne survival of the fungus, local over-wintering of inoculum, cultivationof susceptible varieties and favourable environmental conditions. it is the most destructive disease in India. Phytopathogens from *Alternaria* genus are known to synthesize phytotoxins that dam-age planttissuesandfacilitate colonization(Pedras *etal*., 2011).

The spread and development of alternaria diseasearewidespreadacrossallregionsthatcultivatecruciferousvegetables.Researchindicatessignificant yield losses due to this disease with cabbage experiencing a reduction of 14.3 per centto 17.4 per cent, cauliflower 8.3 per cent to 12.1 per cent, red cabbage 10.0 per cent, napa cabbage10.0 percent to 12.2 percentand broccoli10.2to16.0 percent(Rakhmonov*et al*.,2023).

An attempt is being made, all over the world, to use plant extracts and bio-agents against plantdiseases. Fortunately, some plant extracts have been identified or detected as having reasonableantifungalqualities.Especiallysomeplantextractshavebeenusedeffectivelytocontrol alternariablightdisease.Theplantsextractscanbeusedincontrollingdiseasehavebeenexperiencedbyman sincethedawnofhumancivilization(Kabir*etal*.,2014).

By the time some plants have already been found very effective, in some cases, more than that ofchemical pesticides. Out of the many promising plants, reported garlic (*Allium sativum*), basil(*Ocimumsanctum*)hasbeenselectedtoassesstheirabilityagainst alternariablight(Sreevarshini*etal*.,2019).

With the growing awareness of harmful effects of pesticides, uses of cultural practices, bio agents,plantextracts ofintegrate withlessfungicidalspray isgainingimportancein recentyears.Biological control agents (BCAs)workagainstplantpathogens usinga variety ofmethods,includingcompetitionfornutrients,thereleaseofsecondarymetabolites,antibiosis,andmycoparasitism. Plants, the environment, and the economy all benefit from the use of biologicalcontrol agents. A number of plant species have been reported to possess some natural substances intheir leaves and bulbs, which were toxic to many fungi causing plant diseases. The use of variousbiocontrol agentsandplantextractsisbeingencouraged.Anotherimportant reasonoftheir increased application is the fact that they are eco-friendly. It is now widely recognized as a biorational approach to control alternaria leaf spot diseases.

1. MATERIALS AND METHODS

The details of the materials used and methods followed for various experiment are described here in the following paragraphs. variety plants from the field experiment were carried out at the Central Research Field, Department of Plant Pathology, SHUATS, Prayagraj during ***Rabi***season 2023. The study was laid-out with Randomized Block Design (RBD) with three replications. Three sprays of all treatments were given at an interval of 15 days. Treatments were imposed after appearance of the first disease symptoms. Observations on disease intensity (%) of alternaria leaf spot of broccoli were recorded at 15 days interval, yield (t/ha) and C:B ratio data were obtained after the harvest on physiological maturity.

1.1 Isolation of fungal organism

The broccoli leaves showing typical disease symptoms were collected and brought to the laboratory for isolation of the pathogen. The diseased leaves were first examined for associated fungi by scrapping the diseased portion with the help of needle and observed under microscope. For isolation of fungi small segments of the diseased tissue along with some healthy portions were cut with a sterilized razor and surface sterilized with 1 per cent sodium hypo chloride solution for 30 seconds, rinsed with three changes of sterile distilled water to remove the disinfectant and blotted dry. The sterilizes pieces were then transferred to petri plates containing potato dextrose agar (PDA) under aseptic conditions and incubated at 25°C for 7 days. To obtain sufficient quantity of inoculums, pure cultures were obtained by sub culturing. For this purpose, small bits of fungus were taken at the tip of a sterilized needle and transferred aseptically to the center of fresh PDA medium in petri plates. The plates were incubated for 2 weeks at 25°C in the dark place (Ellis, 1968).

Picture 1. Laboratory analysis

1.2 Evaluation of treatments under field conditions

The efficacy of bio-agents and botanicals against *Alternaria alternata* was carried out in field conditions.

1.3 Management of the disease through bio agents and botanicals

Bio-agents were incorporated in the soil a day, before transplanting of broccoli. Two foliar sprays of botanicals were applied at a interval of 15 days after the first appearance of diseased symptoms.

1.4 Preparation of botanicals

Hundred grams of fresh healthy plant parts (bulbs and leaves) collected from field and washed with distilled water and crushed in 100 ml of distilled water (w/v). The crushed product was filtered through double layer, muslin cloth and further filtrated through Whatsman No. 1 filter paper using funnel and volumetric flasks (100 ml cap.). The prepared solution was 100 per cent concentration, which was further diluted to required concentrations of 10 per cent (Shekhawat and Prasada, 1971).

1.5 Disease intensity (%)

The Percent disease intensity of 5 randomly selected plants was calculated at 50, 70 and 90 DAS. Disease intensity (%) formula is given by Wheeler (1969). It was calculated by using the following formula:

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Disease Intensity was recorded on first appearance of symptoms and after 15 days of 1st and 2nd spray. Leaves of five randomly selected plants of each treatment of each treatments of each replication was observed. The scale for scoring of leaf spot was observed following 0-5 scale Table 1. (Sangeetha and Siddaramaiah, 2007). The disease rating was based on surface of leaf area covered with leaf spot.

Table 1. Description of disease rating scale

Class/Grade	Description
0	No infection
1	<5% infection
2	5-10 % infection
3	10-25 % infection
4	25-50 % infection
5	> 50 % infection

5 4 3 2 1 0

Picture 2. Disease behavior according to rating scale

Table 2. Details of experiment layout

Nameofcrop	Broccoli(*B.oleraceavar.italica*)
Season	*Rabi*(2023)
Variety	PalamSamridhi
Experimentaldesign	RandomBlock Design(RBD)
Numberofreplications	3
Numberoftreatments	8
Totalnumber ofplots	24
Plotsize	2.0×1.0=2.0m^2
Sizeofbunds	0.3m
Widthofmainirrigationchannel	1m
Widthofsub-irrigationchannel	0.5m
Totalgrosscultivatedarea	97.11m^2
Totalnetcultivatedarea	48m^2
SpacingRowtoRow	45-60cm
Planttoplant	45×45cm
Duration	85-95days

Table 3.Treatmentscombination

S.No.	Treatments	TreatmentDetails	Doses	References
1.	**T_0**	Control(Untreated)	-	-
2.	**T_1**	*Pseudomonasfluorescens*	@2%(S.T.)	Sailaja*etal*.(2017)
3.	**T_2**	*Trichodermaviride*	@2%(S.T.)	Sailaja*etal*.(2017)
4.	**T_3**	*Pseudomonasfluorescens*+ *Ocimumsanctum*	@2%(S.T.)+ @10%(F.S.)	Sailaja*et-al*.(2017)+Sreevarshini*etal*.(2019)
5.	**T_4**	*Trichodermaviride*+ *Alliumsativum*	@2%(S.T.)+ @10%(F.S.)	Sailaja*et-al*.(2017)+Meena *etal*.(2022)
6.	**T_5**	*Trichodermaviride*+ *Ocimumsanctum*	@2%(S.T.)+ @10%(F.S.)	Sailaja*et-al*.(2017)+Sreevarshini*etal*.(2019)
7.	**T_6**	*Pseudomonasfluorescens*+ *Alliumsativum*	@2%(S.T.)+ @10%(F.S.)	Sailaja*et-al*.(2017)+Meena *etal*.(2022)
8.	**T7**	Carbenda-zim(Treatedcontrol)	@0.1%(F.S.)	Valvi*etal*.(2019)

Where,

S.T.-SoilTreatment

F.S.-FoliarSpray

RESULTSANDDISCUSSION

The study entitled, “Effect of bio-agents and selected botanicals on alternaria leaf spot caused by*Alternaria alternata* (Fr.) Keissler in broccoli (*Brassica oleracea* var. italica L.)” was carried outin Central Research Field,Departmentof PlantPathology,SHUATS,Prayagrajduring*Rabi*season2023-24.TheexperimentwascarriedoutinRandomizedBlockdesign(RBD).Amongthefungaldiseases,alternarial eafspotofbroccoli caused by *Alternaria alternata* was identified in broccoli. For its management, selectedbio agents and plant-extracts was used and results obtained are being presented and discussed inthischapter.

Table4.Effectoftreatmentsondiseaseintensity(%)ofAlternarialeafspoton broccoliat45,60 and75 DAT

	Treatmentdetails		15 daysafter 1st spray of botanicals	15daysafter 2nd spray of botanicals	15daysafter 3rd spray of botanicals
	Soiltreatment	**Foliarspray**	**45DAT**	**60DAT**	**75DAT**
T_0	Control(untreated)	Waterspray	20.93[a]	29.81[a]	38.31[a]
T_1	*Trichoderma viride*	-	17.39[c]	27.34[b]	33.56[c]
T_2	*Pseudomonas fluorescens*	-	18.62[b]	27.75[b]	34.30[b]
T_3	*Pseudomonas fluorescens*	*Ocimum sanctum*	16.31[d]	25.44[c]	30.68[d]
T_4	*Trichodermaviride*	*Alliumsativum*	14.22[f]	22.39[e]	28.16[f]
T_5	*Trichodermaviride*	*Ocimum sanctum*	15.38[e]	23.58[d]	29.68[e]

T_6	*Pseudomonas fluorescens*	*Alliumsativum*	16.14[d]	26.25[c]	30.44[d]
T_7	Carbendazim(Treatedcontrol)		12.04[g]	20.26[f]	24.42[g]
	SE(m)±1		**0.21**	**0.27**	**0.17**
	CDat5%		**0.64**	**0.82**	**0.53**

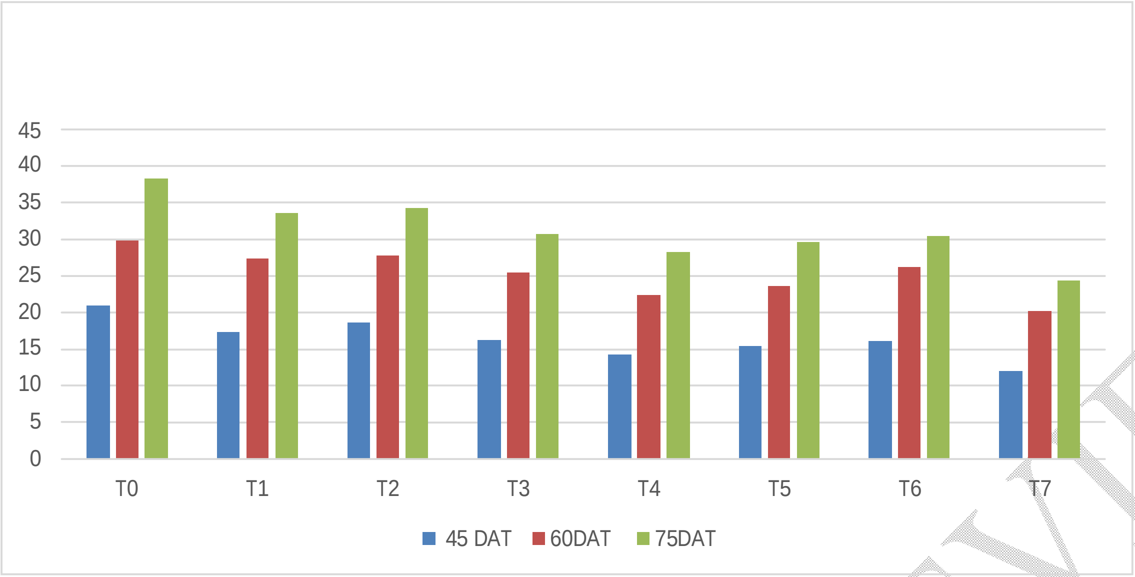

Figure1.Effectoftreatmentsondiseaseintensity(%)ofalternarialeafspot onbrocco-liat45,60and75DAT

1. Diseaseintensity(%) ofAlternarialeafspotonbroccoliat45DAT

ThedatapresentedintheTable4. anddepictedinFigure1. revealedthatdiseaseintensity(%) of broccoli significantly decreased in treatment T_4– *Trichoderma viride* + *Allium sativum*(14.22%) followed by T_5– *Trichoderma viride* + *Ocimum sanctum* (15.38%), T_6 – *Pseudomonasfluorescens* + *Allium sativum* (16.14%), T_3– *Pseudomonas fluorescens* + *Ocimum sanctum*(16.31%) and T_1- *Trichoderma viride* (17.39%) T_2– *Pseudomonas fluorescens* (18.62%) ascompared to(Treatedcontrol)T_7(12.04%)and(Untreated)T_0–(20.93%).

2. Diseaseintensity(%) ofAlternarialeafspotonbroccoliat60DAT

ThedatapresentedintheTable 4. anddepictedinFigure1.revealedthatdiseaseintensity(%)ofbroccolisignificantlydecreasedintreatmentT_4–*Trichodermaviride*+*Alliumsativum* (22.39%) followed by T_5 – *Trichoderma viride* + *Ocimum sanctum* (23.58%), T_6 – *Pseudomonasfluorescens*+ *Allium sativum* (26.25%), T_3– *Pseudomonas fluorescens* + *Ocimum sanctum*(25.44%) and T_1- *Trichoderma viride* (27.34%) T_2– *Pseudomonas fluorescens* (27.75%) ascomparedto(Treatedcontrol)T_7(20.26%)and(Untreatedcontrol)T_0–(29.81%).

3. Diseaseintensity(%) ofAlternarialeafspotonbroccoliat75DAT

ThedatapresentedintheTable4.anddepictedinFigure1.revealedthatdiseaseintensity(%) of broccoli significantly decreased in treatment T_4– *Trichoderma viride* + *Allium sativum*(28.17%) followed by T_5 – *Trichoderma viride* + *Ocimum sanctum* (29.68%), T_6 – *Pseudomonasfluorescens*+ *Allium sativum* (30.44%), T_3– *Pseudomonas fluorescens* + *Ocimum sanctum*(30.68%) and T_1- *Tricho-derma viride* (33.56%) T_2– *Pseudomonas fluorescens* (34.30%) ascompared to(Treatedcontrol)T_7(24.42)and(Untreatedcontrol)T_0–(38.31%).

From the present investigation, it is concluded that all the treatments were significantlysuperior over control and minimum number of spots is observed in treatment T4 − *Trichodermaviride* @ 2% (ST) combined with *Allium sativum* @ 10% as a foliar spray. Similar findings havebeen reported by Devi and zacharia (2024). The probable reason may be due to *T. viride* may have reducedthe severity of plantdisease by inhibiting soil pathogen through its potentantagonistic andmycoparasiticactivities.*T.viride*may haveproducedsecondarymetaboloitessuchasharzianicacid,alamethicins,peptaibols,antibiotics,6-pethyl-α-pyrone,massoilactone,viridin,glioviridin,gliovirin, glisoprenins, heptelidic acid, pentyl pyrone etc which may have shown antifungal properties (Verma*et-al*.,2007).*Alliumsativum*(garlic)thevolatileantimicrobialsubstanceallicin (diallylthiosulphinate) is produced when the tissues are damaged and the substrate alliin (S-allyl-L-cysteine sulphoxide) mixes with the enzyme alliin-lyase (E.C.4.4.1.4). Allicin is readily membrane-permeable and undergoes thiol-disul- phide exchange reactions with free thiol groups in proteins. Itisthoughtthat-theseproperties may have shown itsantimicrobialactionagainst seed-borne*Alternaria*spp. A wide range of secondary metabolites have been identified in *A. sativum* (Slusarenko *et al.*,2007). So, in combination of both *T. viride* (soil application) and *A. sativum* (foliarspray) may have shownsignificantresultsagainst Alternaria leafspotofbroccoli.Themostpotentinhibitionof the pathogen leading to its superiority in all plant parameters including the reduced disease intensity. But amongall the treatments chemical fungicide (Treated control) T_7has shown the highest per cent of disease inhibition withstrongfungicidaleffectagainstpathogen.Inordertoreducethepathogen,itmayproducesometoxicchemicalresidues,theymayhavepotentialharmfuleffectstonon-targetedorganism.So,consideringtheecosystem,bioagent*T.viride*and botanicals of *A.sativum*significantly inhibitsthepathogen,thiscascadeeffect mayhavecontributedtotheholistic-well-beingofplants,consequentlyresulting in reduced disease intensity. Similar findings are consistent with the research conducted byTanwar*etal.*(2013)andSupriya*etal.*(2022).

Benefit Cost ratio

The data on Benefit Cost ratio of the treatments was presented in table 5 and Figure 2.

Table 4.8.EconomicsofCultivation:

Tr. No:	Treatment	Yield t/ha	cost ofyield(Rs/t)	Grossreturn(Rs/ha)	Common cost(Rs)	Treatment cost (Rs)	Total costof cultivation(Rs)	C:B ratio
T_0	Control	6.56	60,000	3,93,600	3,00,649	-	3,00,649	1:1.30
T_1	*Trichodermaviride*	7.74	60,000	4,64,400	3,00,649	1075	3,01,724	1:1.53
T_2	*Psuedomonasfluorescens*	7.04	60,000	4,22,400	3,00,649	1150	3,01,799	1:1.39
T_3	*Psuedomonasfluorescens+ Ocimum mumsanctum*	8.15	60,000	4,89,000	3,00,649	30,250	3,30,899	1:1.47
T_4	*Trichodermaviride +Alliumsativum*	10.37	60,000	6,22,200	3,00,649	24,175	3,24,824	1:1.915
T_5	*Trichodermaviride + Ocimum mumsanctum*	9.41	60,000	5,64,600	3,00,649	30,175	3,30,824	1:1.70
T_6	*Psuedomonasfluorescens+ Alliumsativum*	8.70	60,000	5,22,000	3,00,649	24,250	3,24,899	1:1.60
T_7	Carbendazim	13.52	60,000	8,11.200	3,00,649	1725	3,02,374	1:2.68

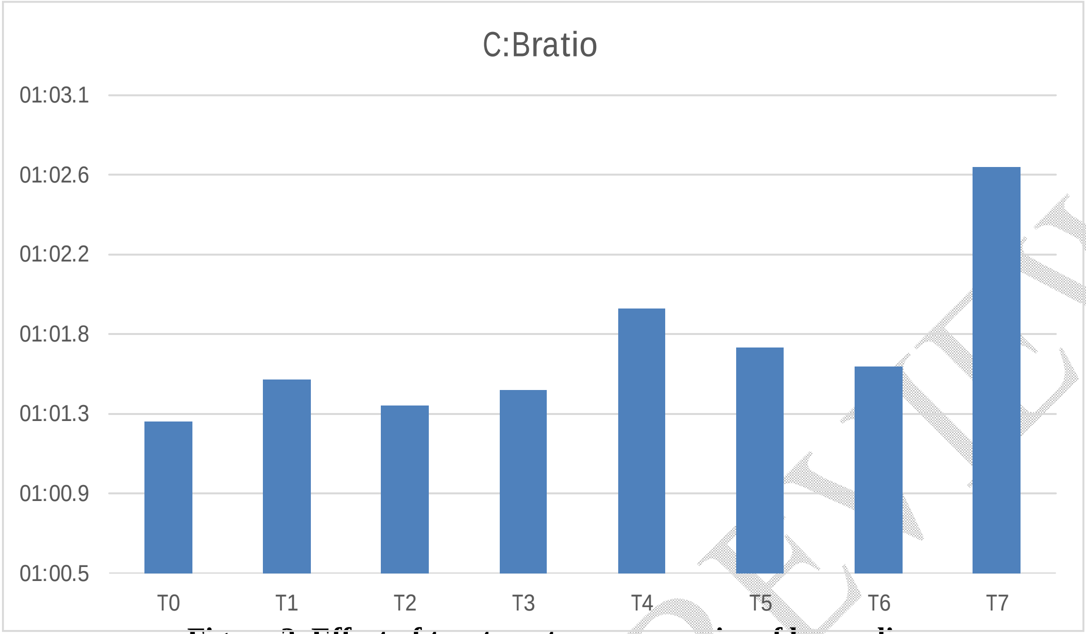

Figure 2. Effect of treatments on economics of broccoli

The treatment wise economics of broccoli production under field demonstrations were estimated and the results have been presented in figure 2. The economics analysis of the data over the session that (*Trichoderma viride* + *Allium sativum*) T4, recorded higher gross returns Rs. 6,22,200, net returns Rs. 2,97,376 with B:C ratio 1:1.91 followed by T5 – *Trichoderma viride* + *Ocimum sanctum* recorded gross returns Rs. 5,64,600, net returns Rs. 2,33,776 with B:C ratio 1:1.70 as compared to (Untreated control) T0 – Control gross returns Rs. 3,93,600, net returns Rs. 92,951 with B:C ratio 1:1.30.

CONCLUSIONS

Alternaria alternata (Fr.) Keissler was found associated with alternaria leaf spot disease ofbroccoli (*Brassica oleracea* var. *italica* L.). The Diseaseintensity (%) and B:C ratio, overallresults revealed that T_4 – *Trichoderma viride* (ST) @ 2% combined with *Allium sativum* (FS)bulb extract @ 10% is significantly effective against *A. alternata* as compared to (Treatedcontrol) T_7 - Carbendazim @ 0.1 % and (Untreated control) T_0. Disease intensity (%) 45 DAT,60 DAT and 75 DATand B: C ratio. It is worth mentioning that the conclusionsdrawnfromthisStudywerebasedonobservationscarriedoutunderfieldconditionsatCentralResearchField(CRF),DepartmentofPlantPathology,SHUATS, Prayagraj, during ***Rabi***Season 2023, within the agro- climatic conditions of Prayagraj, UP. India. As such, furtherresearchandmoreexperimentationovermanyseasonsshouldbeconductedinfuturetovalidatethe presentfindingsandforfurtherrecommendations.

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