

Original Research Article

Effectsofbio-agentsandselectedbotanicalsonAlternarialeafspotcausedby *Alternariaalternata*(Fr.)Keisslerinbroccoli(*Brassicaoleraceavar.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., T₁– (*Pseudomonas fluorescens* (S.T) @ 2%, T₂– *Trichoderma viride* (S.T) @ 2%, T₃– *Pseudomonas fluorescens* (S.T) @ 2% + *Ocimum sanctum* (F.S) @ 10 %, T₄– *Trichoderma viride* (S.T) @ 2 % + *Allium sativum* (F.S) @10%, T₅– *Trichoderma viride* (S.T) @ 2% + *Ocimum sanctum* (F.S) @ 10%, T₆ - *Pseudomonas fluorescens* (S.T) @ 2% + *Allium sativum* (F.S) @ 10 %, T₇ – Carbendazim @ 0.1% (F.S) (treated control) and control T₀(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 per cent disease intensity (%) was recorded in T₄ –28.17% *Trichodermaviride*@ 2% (S.T) combined with *Allium sativum* @ 10% (F.S)as compared with the untreated control T₀ – 38.31 % and treated check T₇ – 24.42 % (Carbendazim @ 0.1% (F.S). PDI recorded in broccoli with maximum cost benefit ratio per treatment was recorded 1:1.915 in T₄ - *Trichodermaviride*@ 2% (S.T) combined with *Allium sativum* @ 10% (F.S)in broccoli.

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

INTRODUCTION

Broccoli (*Brassica oleracea* var. *italica* L.) is an important and highly exotic and nutritious vegetable. It is a member of the cruciferous family originated from Italy about more than 2000 years ago (Buck, 1956). It is also known as winter

broccoli and heading broccoli. It is considered to be originated from wild 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 a thick, edible stalk. Broccoli is a high-quality vegetable for fresh use and is one of the most popular frozen vegetables. It is also used as a vegetable in many other countries, such as Spain, Mexico, Italy, France and the United States (Pate *et al.*, 2023).

It is a winter season crop and grown as biennial crop for seed production, but in tropical and subtropical areas it is mainly grown as annual vegetable crop. For broccoli production, the ideal temperature required 25°C to 26°C during the day and 16°C to 17°C at night (Ferdinand *et al.*, 2022).

The demand

for broccoli increases worldwide by 4 percent annually due to its essential health benefits. Specifically, it has been reported to have a beneficial effect against various types of cancer, such as lung, prostate and breast, since its high content of antioxidant compounds like carotene (1.9 mg carotene per 100 g of fresh broccoli). Nutritionally, it is rich in vitamin-A (2500 I.U.), vitamin C (113 mg), protein (3.6 g), carbohydrates (5.9 g) and minerals like calcium (103 mg), iron (1.1 mg), phosphorus (78 mg), potassium (382 mg) and sodium (15 mg) per 100 g of an edible portion (USDA, 2023).

The leading producers of broccoli are China with 36.83 percent, India with 36.74 percent, the United States of America (US) with 3.56 percent, Mexico with 2.84 percent and Spain with 2.60 percent, others with 18 percent (FAO, 2023).

The major broccoli diseases are damping off, club root of crucifers or finger and toe disease, alternaria leaf spot, black rot, downy mildew and white rust. Alternaria leaf spot is one of the factors responsible for economic losses in broccoli (Lafi *et al.*, 2022).

Among all the diseases alternaria leaf spot caused by *Alternaria* sp. has become a problematic disease due to the soil-borne survival of the fungus, local over-wintering of inoculum, cultivation of susceptible varieties and favourable environmental conditions. It is the most destructive disease in India. Phytopathogens from *Alternaria* genus are known to synthesize phytotoxins that damage plant tissues and facilitate colonization (Pedraza *et al.*, 2011).

The spread and development of alternaria disease are widespread across all regions that cultivate cruciferous vegetables. Research indicates significant yield losses due to this disease with cabbage experiencing a reduction of 14.3 per cent to 17.4 per cent, cauliflower 8.3 per cent to 12.1 per cent, red cabbage 10.0 per cent, napa cabbage 10.0 per cent to 12.2 per cent and broccoli 10.2 to 16.0 per cent (Rakhmonov *et al.*, 2023).

An attempt is being made, all over the world, to use plant extracts and bio-agents against plant diseases. Fortunately, some plant extracts have been identified or detected as having reasonable antifungal qualities. Especially some plant extracts have been used effectively to control alternaria blight disease. The plant extracts can be used in controlling disease have been experienced by mankind since the dawn of human civilization (Kabir *et al.*, 2014).

By the time some plants have already been found very effective, in some cases, more than that of chemical pesticides. Out of the many promising plants, reported garlic (*Allium sativum*), basil (*Ocimum sanctum*) has been selected to assess their ability against alternaria blight (Sreevarshini *et al.*, 2019).

With the growing awareness of harmful effects of pesticides, uses of cultural practices, bio agents, plant extracts of integrate with less fungicidal spray is gaining importance in recent years. Biological control agents (BCAs) work against plant pathogens using a variety of methods, including competition for nutrients, the release of secondary metabolites, antibiosis, and mycoparasitism. Plants, the environment, and the economy all benefit from the use of biological control agents. A number of plant species have been reported to possess some natural substances in their leaves and bulbs, which were toxic to many fungi causing plant diseases. The use of various bio-control agents and plant extracts is being encouraged. Another important reason for their 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 experiments are described here in the following paragraphs. Various 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 sterilized 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 centre 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 an 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 Whatman 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:

Sum of all disease ratings

Disease intensity (%) = x 100

Total number of ratings × Maximum disease grade

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

Name of crop	Broccoli (<i>B. oleracea</i> var. <i>italica</i>)
Season	Rabi (2023)
Variety	Palam Samridhi
Experimental design	Random Block Design (RBD)
Number of replications	3
Number of treatments	8
Total number of plots	24
Plot size	2.0 × 1.0 = 2.0 m ²
Size of bunds	0.3 m
Width of main irrigation channel	1 m
Width of sub-irrigation channel	0.5 m
Total gross cultivated area	97.11 m ²
Total net cultivated area	48 m ²
Spacing Row to Row	45-60 cm
Plant to plant	45 × 45 cm
Duration	85-95 days

Table3.Treatmentscombination

S.No.	Treatments	TreatmentDetails	Doses	References
1.	T₀	Control(Untreated)	-	-
2.	T₁	<i>Pseudomonasfluorescens</i>	@2%(S.T.)	Sailajaetal.(2017)
3.	T₂	<i>Trichodermaviride</i>	@2%(S.T.)	Sailajaetal.(2017)
4.	T₃	<i>Pseudomonasfluorescens</i> + <i>Ocimumsanctum</i>	@2%(S.T.)+ @10%(F.S.)	Sailajaetal.(2017)+Sre varshinietal.(2019)
5.	T₄	<i>Trichodermaviride</i> + <i>Alliumsativum</i>	@2%(S.T.)+ @10%(F.S.)	Sailajaetal.(2017)+M eena etal.(2022)

6.	T ₅	<i>Trichoderma viride</i> + <i>Ocimum sanctum</i>	@2%(S.T.)+ @10%(F.S.)	Sailaja <i>et al.</i> (2017)+Sre evarshini <i>et al.</i> (2019)
7.	T ₆	<i>Pseudomonas fluorescens</i> + <i>Allium sativum</i>	@2%(S.T.)+ @10%(F.S.)	Sailaja <i>et al.</i> (2017)+M eena <i>et al.</i> (2022)
8.	T ₇	Carbendazim(T reated control)	@0.1%(F.S.)	Valvi <i>et al.</i> (2019)

Where,

S.T.-Soil Treatment

F.S.-Foliar Spray

RESULTS AND DISCUSSION

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 out in Central Research Field, Department of Plant Pathology, SHUATS, Prayagraj during *Rabi* season 2023-24. The experiment was carried out in Randomized Block design (RBD). Among the fungal diseases, alternaria leaf spot of broccoli caused by *Alternaria alternata* was identified in broccoli. For its management, selected bio agents and plant-extracts was used and results obtained are being presented and discussed in this chapter.

Table 4. Effect of treatments on disease intensity (%) of Alternaria leaf spot on broccoli at 45, 60 and 75 DAT

	Treatment details		15 days after 1 st spray	15 days after 2 nd spray	15 days after 3 rd spray
	Soil treatment	Foliar spray			

			of botanicals	botanicals	botanicals
			45DAT	60DAT	75DAT
T ₀	Control(untreated)	Waterspray	20.93 ^a	29.81 ^a	38.31 ^a
T ₁	<i>Trichoderma viride</i>	-	17.39 ^c	27.34 ^b	33.56 ^c
T ₂	<i>Pseudomonas fluorescens</i>	-	18.62 ^b	27.75 ^b	34.30 ^b
T ₃	<i>Pseudomonas fluorescens</i>	<i>Ocimum sanctum</i>	16.31 ^d	25.44 ^c	30.68 ^d
T ₄	<i>Trichoderma viride</i>	<i>Allium sativum</i>	14.22 ^f	22.39 ^e	28.16 ^f
T ₅	<i>Trichoderma viride</i>	<i>Ocimum sanctum</i>	15.38 ^e	23.58 ^d	29.68 ^e
T ₆	<i>Pseudomonas fluorescens</i>	<i>Allium sativum</i>	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

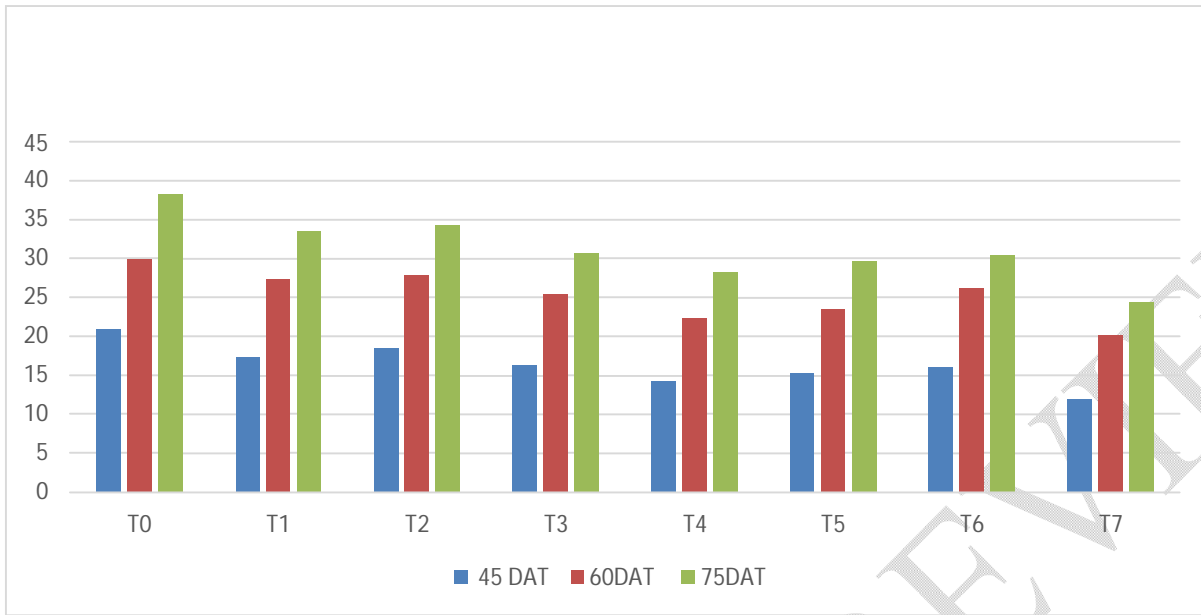


Figure 1. Effect of treatments on disease intensity (%) of Alternaria leaf spot on broccoli at 45, 60 and 75 DAT

1. Disease intensity (%) of Alternaria leaf spot on broccoli at 45 DAT

The data presented in the Table 4, and depicted in Figure 1, revealed that disease intensity (%) of broccoli significantly decreased in treatment T₄– *Trichoderma viride* + *Allium sativum* (14.22%) followed by T₅– *Trichoderma viride* + *Ocimum sanctum* (15.38%), T₆ – *Pseudomonas fluorescens* + *Allium sativum* (16.14%), T₃– *Pseudomonas fluorescens* + *Ocimum sanctum* (16.31%) and T₁– *Trichoderma viride* (17.39%) T₂– *Pseudomonas fluorescens* (18.62%) as compared to (Treated control) T₇ (12.04%) and (Untreated) T₀– (20.93%).

2. Disease intensity (%) of Alternaria leaf spot on broccoli at 60 DAT

The data presented in the Table 4, and depicted in Figure 1, revealed that disease intensity (%) of broccoli significantly decreased in treatment T₄– *Trichoderma viride* + *Allium sativum* (22.39%) followed by T₅ – *Trichoderma viride* + *Ocimum sanctum* (23.58%), T₆ – *Pseudomonas fluorescens* + *Allium sativum* (26.25%), T₃– *Pseudomonas fluorescens* + *Ocimum sanctum* (25.44%) and T₁– *Trichoderma viride* (27.34%) T₂– *Pseudomonas fluorescens* (27.75%) as compared to (Treated control) T₇ (20.26%) and (Untreated control) T₀– (29.81%).

UNDER PEER REVIEW

3. Disease intensity (%) of *Alternaria* leaf spot on broccoli at 75 DAT

The data presented in the Table 4 and depicted in Figure 1 revealed that disease intensity (%) of broccoli significantly decreased in treatment T₄ – *Trichoderma viride* + *Allium sativum* (28.17%) followed by T₅ – *Trichoderma viride* + *Ocimum sanctum* (29.68%), T₆ – *Pseudomonas fluorescens* + *Allium sativum* (30.44%), T₃ – *Pseudomonas fluorescens* + *Ocimum sanctum* (30.68%) and T₁ – *Trichoderma viride* (33.56%) T₂ – *Pseudomonas fluorescens* (34.30%) as compared to (Treated control) T₇ (24.42) and (Untreated control) T₀ (38.31%)

From the present investigation, it is concluded that all the treatments were significantly superior over control and minimum number of spots is observed in treatment T₄ – *Trichoderma viride* @ 2% (ST) combined with *Allium sativum* @ 10% as a foliar spray. Similar findings have been reported by **Devi and zacharia 2024**. The probable reason may be due to *T. viride* may have reduced the severity of plant disease by inhibiting soil pathogen through its potent antagonistic and mycoparasitic activities. *T. viride* may have produced secondary metabolites such as harzianic acid, alamethicins, peptaibols, antibiotics, 6-pethyl- α -pyrone, massoialactone, viridin, glioviridin, gliovirin, glisoprenins, heptelidic acid, pentyl pyrone etc which may have shown antifungal properties (**Verma et al., 2007**). *Allium sativum* (garlic) the volatile antimicrobial substance allicin (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-disulphide exchange reactions with free thiol groups in proteins. It is thought that these properties may have shown its antimicrobial action against 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* (foliar spray) may have shown significant results against *Alternaria* leaf spot of broccoli. The most potent inhibition of the pathogen leading to its superiority in all plant parameters including the reduced disease intensity. But among all the treatments chemical fungicide (Treated control) T₇ has shown the highest per cent of disease inhibition with strong fungicidal effect against pathogen. In order to reduce the pathogen, it may produce some toxic chemical residues, they may have potential harmful effects on non-targeted organism. So, considering the ecosystem, bioagent *T. viride* and botanicals of *A. sativum* significantly inhibit the pathogen, this cascade effect may have contributed to the holistic well-being of plants, consequently resulting in reduced disease

intensity. Similar findings are consistent with the research conducted by Tanwar *et al.* (2013) and Supriya *et al.* (2022).

Benefit Cost ratio

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

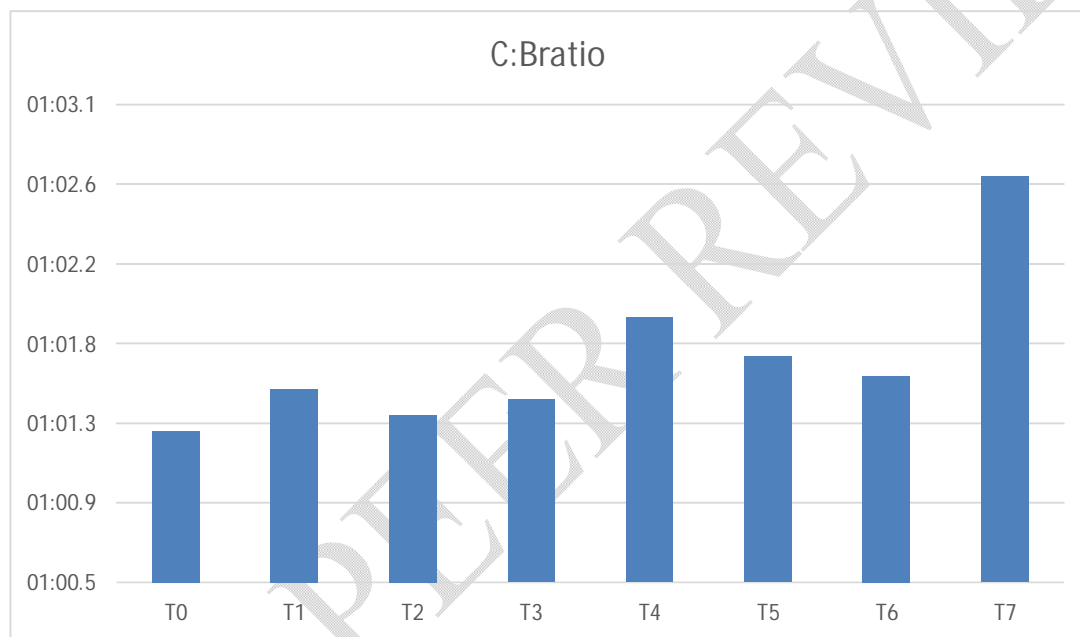


Figure 2. Effect of treatments on economics of broccoli

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.

REFERENCES

- i. **Buck, P.A. (1956).** Origin and taxonomy of broccoli. *Economic Botany*. 10:250-253.
- ii. **Ferdinand, Y. and Al - Maki, W. F. (2022).** Broccoli leaf diseases classification using support vector machine with particle swarm optimization based on feature selection. *International Journal of Advances in Intelligent Informatics*. 8(3): 337-348.
- iii. **Devi, K. P., and Zacharia, S. (2024).** 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. *Journal of Advances in Biology & Biotechnology*. 27(9): 1212-1222.
- iv. **Patel, A., Chandra, R. and Upadhyay, M. (2018).** Efficacy of different fungicides against *Alternaria brassicae* caused alternaria leaf spot of cauliflower. *The Pharma Innovation Journal*. 7(12):231-234.
- v. **Lafi, O.I.A., El-Hamarnah, H.A., Al-Saloul, N.J.H., Radwan, H.I.A. and Abu-Naser, S.S. (2022).** A proposed expert system for broccoli diseases diagnosis. *International Journal of Engineering and Information Systems*. 6(5):43-51.
- vi. **Pedras, M. S. C., Yaya, E. E. and Glawischnig, E. (2011).** The phytoalexins from cultivated and wild crucifers: Chemistry and biology. *Natural Product Reports*. 28(8):1381.
- vii. **Rakhmonov, U., Allayarov, A., and Rakhmonov, U. (2023).** Alternaria disease of cruciferous vegetable crops. *BIO Web of Conferences*. 65: 01023.
- viii. **Rakhmonov, U., Allayarov, A., and Rakhmonov, U. (2023).** Alternaria disease of cruciferous vegetable crops. *BIO Web of Conferences*. 65: 01023.
- ix. **Sreevarshini, D., Kumar, V.S., Simon, S. and Lal, A.A. (2019).** Effect of selected botanicals against leaf spot (*Alternaria brassicae*) of cabbage (*Brassica oleracea* var. *capitata* L.). *International Journal of Current Microbiology and Applied Sciences*. 8(12):1426-1429.
- x. **Ellis, M. B. (1971).** Dematiaceous Hyphomycetes. *Common Wealth Mycological Institute*. 464 -497.
- xi. **Meena, S., Godika, S., Ghasolia, R. P., Sumitra, Nitharwal, N. and Kardam, V. K. (2022).** Management of alternaria blight disease (*Alternaria brassicae*) of mustard through plant extracts and fungicides. *The Pharma Innovation Journal*. 11(1): 58-67.
- xii. **Sailaja, S., Lal, A.A. and Simon, S. (2017).** Comparative efficacy of bioagents, botanicals and fungicides against alternaria leaf spot (*Alternaria brassicae*) of cauliflower (*Brassica*

- oleraceavar. botrytis*). *Journal of Pharmacognosy and Phytochemistry*. 6(5):519-521.
- xiii. **Shekhawat, P. S. and Prasada, R. L. (1971)**. Antifungal activities of some plant extracts. Inhibition of spore germination. *Indian Phytopathology*. 24: 800-802.
- xiv. **Sangeetha, C. G. and Siddaramaiah, A. L. (2007)**. Epidemiological studies of white rust, downy mildew and alternaria blight of Indian mustard. *African Journal of Agricultural Research*. 2(7):305-308.
- xv. **Slusarenko, A. J., Patel, A. and Portz, D. (2008)**. Control of plant diseases by natural products, allicin from garlic as a case study. *European Journal of Plant Pathology*. 121: 313–322.
- xvi. **Supriya, V., Tiwari, S., Sindu, P. L. and Govardhani, P. (2022)**. Effect of selected bio-agents and botanicals on alternaria leaf spot of coriander (*Coriandrum sativum* L.) caused by *Alternaria alternata* (Fries) Keissler. *International Journal of Plant and Soil Science*. 34(23):1532-1537.
- xvii. **Tanwar, A., Aggarwal, A., Kaushish, S. and Chauhan, S. (2013)**. Interactive effect of fungi with *Trichoderma viride* and *Pseudomonas fluorescens* on growth and yield of broccoli. *Plant Protection Science*. 49:137-145.
- xviii. **Valvi, H. T., Saykar, A. D. and Bangar, V. R. (2019)**. *In vitro* and *in vivo* field efficacy of different fungicides against *Alternaria brassicae* (Berk.) Sacc. causing alternaria leaf spot of cauliflower. *Journal of Pharmacognosy and Phytochemistry*. 8(2):1333-1337.
- xix. **Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y. and Valero, J. R. (2007)**. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochemical Engineering Journal*. 37(1): 1–20.
- xx. **Wheeler, B. J. (1969)**. An Introduction of Plant Disease. John Wiley and Sons. Ltd., London. pp.301