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Eco-friendly management of *Alternaria* leaf spot disease of brinjal in Kashmir

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Abstract
Alternaria leaf spot caused by *Alternaria alternata* is one of the important foliar diseases of brinjal (*Solanum melongena* L.). The study focused on sustainable and eco-friendly management of *Alternaria* leaf spot of brinjal in Kashmir valley. The pathogen associated with the disease was identified as *Alternaria alternate*. The efficacy of various bio-agents and plant extracts was carried out *in vitro* and *Trichoderma harzianum* showed highest inhibition among bioagents and among plant extracts Garlic bulb extract showed maximum mycelial growth inhibition. Eco-friendly disease management in the field with most effective bio-agents and plant extracts evaluated *in vivo* showed that seed treatment with *T. harzianum* and three foliar spray with Garlic clove extract could effectively manage the disease with lowest disease incidence of 19.13 per cent and intensity of 9.02 per cent respectively and highest fruit yield of 197.80 q/ha, as compared to control plot showing highest disease incidence of 65.51 per cent and intensity of 34.51 per cent respectively with lowest fruit yield of 106.46 q/ha.

Keywords: Eco-friendly, management, brinjal leaf spot, *Alternaria alternate*, bioagents, plant extract

1. Introduction
Brinjal (*Solanum melongena*.L) belongs to family Solanaceae, is an important and indigenous vegetable crop of India. It is a very good source of protein, dietary fiber and highly productive and usually finds a place as "poor man's crop" (Rajan and Markose, 2002) [20]. Brinjal crop is attacked by number of fungal, bacterial, viral and phytoplasmal diseases like *Alternaria* leaf spot (*Alternaria alternata* (Fr.) Keissler), Damping off (*Pythium aphanidermatum Phytophthora spp*.; *Rhizoctonia spp*.), Phomopsis blight (*Phomopsis vexans*), Cercospora leaf spot (*Cercospora solanimelongenae*, *C.solani*.), Fusarium wilt (*Fusarium solani*, Bacterial wilt (*Ralstonia solanacearum*), little leaf (Phytoplasma.). *Alternaria* leaf blight and fruit rot diseases is most severe disease of brinjal and appears regularly, causing heavy losses in yield (Balai and Ahir, 2013) [3]. The disease first makes its appearance in young seedling. It attacks leaves and then spreads to fruits which subsequently rot and become unfit for consumption (Bochalya et al., 2012) [4]. In Kashmir the disease incidence and intensity of 30.76 and 9.77 per cent, respectively was reported in a study 2017 (Raina *et al*., 2018) [18]. The negative effects of pesticides, the environmental risks resulting from their indiscriminate use and development of resistance in pathogens have renewed interest towards use of sustainable and ecofriendly approaches for the management of plant diseases like use of bio-control agents and plant extracts. Further pesticides have some direct harmful effect on plants including poor root hair development, shoot yellowing and reduced plant growth (Wally *et al*., 2006) [28]. Hence, effect of plant extracts and bioagents, individually and in combination, was evaluated in this study for integrated management of *Alternaria* leaf spot of brinjal.

2. Material and Method
2.1 Isolation of Pathogen
Brinjal leaves depicting the *Alternaria* leaf spot symptoms (Plate1: A, B) were used for isolation of the pathogen. Small segments of size 2-3 mm diseased leaf tissue along with some healthy portion were cut and surface sterilized in 0.1 per cent mercuric chloride solution for 30 seconds. The segments were then rinsed thrice in sterilized distilled water to remove the traces of mercuric chloride solution, blotted dry and placed on acidified potato dextrose agar (PDA) medium in sterilized petri-plates under aseptic conditions. The inoculated PDA plates were incubated at 25±1℃ for 7 days and then sub cultured onto fresh PDA medium.

2.2 Pathogenicity test
Sterilized soil in the pots was used to raise the seedlings of brinjal cultivar "Pusa purple long", susceptible to *Alternaria* leaf spot disease for pathogenicity test.

Seedling were inoculated with conidial suspension of the *A. alternata* (1x10^6 conidia /ml) at 4-6 leaf stage by spraying suspension on both sides of the foliage with help of an atomizer. Under greenhouse conditions, these plants were regularly observed for development of symptoms.

2.3 Evaluation of bio-agents and plant extracts *in-vitro*

2.3.1 Bio-agents

The antagonistic activity of bio-control agents, viz., *Trichoderma viride* Pers., *Trichoderma harzianum* Rifai, *Pseudomonas fluorescens* (Trevisan) Migula and *Bacillus subtilis* (Ehrenberg) Cohn, were evaluated against *A. alternata* by using dual culture method (Dennis and Webster, 1971) [5]. Culture discs (5 mm) of each bio-control antagonist and the pathogen were taken from the actively growing cultures and transferred to potato dextrose agar (PDA) medium contained in 90 mm petriplates on opposite side, approximately at 10 mm from the wall of the plate. Similarly, bacteria were streaked on the opposite side of the pathogen. A check having the test pathogen only was kept for comparison. The experiment was conducted in completely randomized design (CRD) with three replications. Observations on antagonistic activity after every 24hr for 5days were recorded and per cent growth inhibition of the test pathogen over control was calculated according to the formula given Vincent (1927) [26] as:

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

Where,
I = Percent Inhibition; C= Colony diameter;
T= Colony diameter in treatment.

2.3.2 Plant extracts

The healthy plant parts of each species were collected and washed with sterile water, semi dried under shade. The semi dried plant extracts were chopped and homogenized with the help of pestle and mortar at the rate of 10 g tissue by adding equal amount (10 ml) of sterilized distilled water (1:1w/v). The extract was filtered through whatman No1 Filter Paper and centrifuged at 2000 rpm for 30 minutes at ambient temperature (26 ±2°C). This supernatant was used as standard plant extract solution (100%). The plant extracts sterilized by autoclaving at 15 psi for 20 minutes were tested at 10 per cent concentration (poisoned food technique, Schimtz, 1930) [22]. The experiment was conducted in completely randomized design (CRD) with three replications. The inoculated petriplates were incubated at 25±1°C in the laboratory. The colony diameters were measured after 7 days in control plates. Per cent inhibition of growth was calculated by using formula given by Vincent (1927) [26] as mentioned above.

2.4 Evaluation of Bio-agents and plant extracts *in-vivo*

The most effective bio-agents and most effective plant extracts evaluated *in vitro* were further evaluated under field conditions against *Alternaria* leaf spot of brinjal. Field trial on eco-friendly management of the disease was conducted at Faculty of Agriculture, SKUAST-K Wadura. The experiment was laid in Randomized complete block design with 2.4m x 2.4m plot size, each plot having 16 plants and replicated thrice, following package of practices by SKUAST-K. The Brinjal seeds of susceptible variety Pusa Purple Long were sown after treatment with the bio-agents. The plant extracts were sprayed thrice at 14 days interval starting from first appearance of disease @10 per cent. Control plots were sprayed with water. The details of treatments imposed in trial are here as under:

Details of Treatments

Treatment No.	Treatment
T_1	Control
T_2	Seed treatment with *T. harzianum*@ 10^8conidia/ml
T_3	Seed treatment with *T. viride* 10^8conidia/ml
T4	Foliar spray with garlic clove extract @10%
T_5	Seed treatment with *T. harzianum* @ 10^8conidia/ml +Foliar spray with garlic clove extract @10%
T_6	Seed treatment with *T. viride* @ 10^8conidia/ml + Foliar spray with garlic clove extract @10%
T_7	Foliar spray with Datura leaf extract @10%
T_8	Seed treatment with *T. harzianum* @ 10^8conidia/ml + Foliar spray with 10% Datura leaf extract @10%
T 9	Seed treatment with *T. viride* @ 10^8conidia/ml + Foliar spray with Datura leaf extract @10%
T_{10}	Foliar spray with Nettle leaf extract @10%
T_{11}	Seed treatment with *T. harzianum* @ 10^8conidia/ml + Foliar spray with 10% Nettle leaf extract @10%
T_{12}	Seed treatment with *T. viride* @ 10^8conidia/ml + Foliar spray with Nettle leaf extract @10%

Observations on disease intensity were recorded using modified 0-5 disease rating scale (Pandey and Pandey 2001) [17] at flowering stage and fruit yield data was also recorded on maturity.

3. Results

3.1 Morphological characters of Pathogen

The pathogen isolate was cultured from naturally infected brinjal plants and the morphological and cultural characters were studied (Plat 2: A-C). The fungus covered 90 mm diameter of petri-plates in about 10 days, however sporulation occurred only under dark conditions. In around fifteen days, when abundant sporulation occurred. The pathogen was identified on the basis of the cultural and morphological characteristic observed under compound microscope and also using taxonomic monograph "*Alternaria* redefined". To confirm species identity, culture was also identified by Indian Type Culture Collection (ITCC), New Delhi where its identity was confirmed as *Alternaria alternata*. Microscopic observations of the isolated fungus revealed that the mycelium was septate, with hyaline hyphae when observed singly and irregularly branched, measuring 2.7-4.4 µm in width. The conidiophores were dark brown in color, septate, branched and measured 34.0-93.0 µm in length and 3.4-9.0 µm in width. Conidia were slightly brown in color spindle shaped with round base and tapering apex measuring 21.8 - 95.9 µm in length and 8.0 -17.0 µm in width having 0-2 longitudinal and 2-6 transverse septa. Conidia were mostly in

chains of 3-12 conidia (Plate-2: C). Microscopic examination also revealed that the colony was spreading, hairy and gray-brown to black in color, mycelium was branched, septate, dark coloured with tints of olive brown whereas conidiophores were septate, short, simple, straight or flexuous dark coloured and conidia were long beaked, muriform, dark coloured, borne in chains, both longitudinal and transverse septa in mature conidia.

3.2 *In vitro* evaluation of Bio-agents and Plant extracts against the pathogen

3.2.1 Bio-agents

Two fungal bio-agents viz., *Trichoderma viride*, *Trichoderma harzianum*, and two bacterial bio-agents *Pseudomonas fluorescens* and *Bacillus subtilis* were screened under *in vitro* conditions against *A. alternata* for their antagonistic activity by dual culture technique. It is apparent from the data presented (Table-1) that all the antagonistic fungi and bacteria inhibited the growth of *A. alternata* ranging from 51.21 to 83.73 per cent. *T. harzianum* was found to be superior over all treatments with 83.73 per cent growth inhibition (Plate-7) followed by *T. viride* (78.45%) and *P. fluorescens* (63.00%), while *B. subtilis* (51.21%) was found to be least effective.

Table 1: *In vitro* efficacy of various bio-agents against mycelia growth inhibition of *Alternaria alternata.*

S. No	Bio-agent	Per cent inhibition of mycelial growth
1	*Trichoderma harzianum*	83.73 (66.20)
2	*Trichoderma viride*	78.45 (62.32)
3	*Pseudomonas fluorescens*	63.00 (52.54)
4	*Bacillus subtilis*	51.21 (45.67)
	C.D ($p \leq 0.05$)	7.296

*Figures in parenthesis are angular transformed values

3.2.2 Plant extracts

Data on Antifungal activity of eight plant extracts against the mycelial growth of *A. alternata* revealed that all the plant extracts were significantly superior in inhibiting the mycelial growth of the fungus over control (Table-2). Among the plant extracts tested, Garlic clove (*Allium sativum*) extract @ 10 per cent concentration caused maximum inhibition of growth (80.22%), followed by leaf extract of *Datura stramonium* @ 10 per cent (63.11%), leaf extract of Nettle (*Utrica dioica L.)* @ 10 per cent (56.67%), Leaf extract of Mint (*Mentha arvensis.*) @ 10 per cent (50.55%), leaf extract of Artimesia (*Artimesia annua)* @ 10 per cent (46.67%) and Green husk extract of Walnut (*Juglens regia)* @ 10 per cent (39.44%), while Onion bulb (*Allium cepa*) extract @ 10 per cent (32.22%) and Leaf extract of Mayweed (*Anthemus* cotula) @ 10 per cent (23.89%), were least effective respectively (Plate-8).

Table 2: *In vitro* efficacy of various plant extracts on mycelial growth inhibition of *Alternaria alternata*

S. No.	Plant species	Plant Part used	Conc. (%)	Percent Inhibition of mycelial growth
1	*Utrica dioica L*	Leaf	10%	56.67 (48.83)*
2	*Datura stramonium*	Leaf	10%	63.11 (52.61)
3	*Jugulens regia*	Green husk	10%	39.44 (38.89)
4	*Mentha arvensis*	Leaf	10%	50.55 (45.30)
5	*Artimesia annua*	Leaf	10%	46.67 (43.07)
6	*Allium sativum*	Clove	10%	80.22 (63.61)
7	*Allium cepa*	Bulb	10%	32.22 (34.55)
8	*Anthemis cotula*	Leaf	10%	23.89 (29.09)
C.D ($p \leq 0.05$):				5.34

3.3 *In vivo* evaluation of Bio-agents and Plant extracts

The bio-agents and plant extracts which are higly effective under *in vitro* against *A. Alternate* were further assessed under field conditions against *Alternaria* leaf spot of brinjal. The treatments comprised of two bio-agents bio-agents (*Trichoderma harzianum* and *Trichoderma viride*) and three plants extract (Garlic clove, Datura and nettle leaf).

Data revealed that all the treatments had significantly reduced per cent disease incidence and intensity of the disease over check. Among the plant extracts Garlic clove extract @10 per cent proved to be most effective as it reduced *Alternaria* leaf spot incidence (32.96%) and intensity (14.21%) compared to check exhibiting leaf spot incidence (65.51%) and intensity (34.51%), followed by Datura leaf extract, Nettle leaf extract exhibiting leaf spot incidence(37.89, 40.38%) and intensity (21.31,23.21%) respectively (Table-3, Figure:1).

Among the bio-agents used as seed treatment *T. harzianum* proved to be most effective over check exhibiting leaf spot incidence(51.73%) and intensity (21.72%) followed by *T. viride* which exhibited leaf spot incidence (58.57%) and intensity (25.54%). A significant combination existed between the test plant extracts and bio-agents. Among the interactions foliar spray of Garlic clove extract @10 per cent + seed treatment with *T. harzianum* proved to be most effective over check exhibiting leaf spot incidence (19.13%) and intensity (9.02%) which was at par with other treatments. Moreover, Datura leaf extract @10 per cent + seed treatment with *T.* viride (29.79%) was statistically at par with Nettle leaf extract @10 per cent + seed treatment with *T. viride* (29.77%). The range of disease incidence (19.13-65.51%) and intensity (9.02-34.51%) in treatments with minimum disease incidence (19.13%) and intensity (9.02%) was recorded in treatment T5, i.e., Seed treatment with *T. harzianum* @ 10^8conidia/ml +Foliar spray with Garlic clove extact @10 per cent.

Data on fruit yield revealed that all the treatments had significant effect on fruit yield of brinjal over check. Among the plant extracts, foliar spray with Garlic clove extract @10 per cent proved to be most effective as the average fruit yield was recorded to 134.82 q/ha indicating an increase of 26.63 per cent compared to check exhibiting on an average fruit yield to 106.46 q/ha, followed by leaf extract treatments.

Among the bio-agents *T. harzianum* proved to be most effective over check as the average fruit yield was recorded to 134.42q/ ha indicating an increase of 26.26 per cent compared to check followed by *T. viride* exhibiting on an average fruit yield to 124.82 q/ha indicating an increase of 17.24 per cent. Among the interactions Garlic clove extract @10 per cent + seed treatment with *T. harzianum* proved to be most effective over check exhibiting on an average fruit yield to 197.80 q/ha followed by Garlic clove extract @10 per cent + *T. viride* (179.20 q/ha) and Datura leaf extract @10 per cent + seed treatment with *T. harzianum* (166.50 q/ha) indicating an increase of 85.58, 68.32and 56.39 per cent, respectively. Datura leaf extract+ *T. viride* exhibited on an average yield of 160.78 q/ha followed by Nettle leaf extract+ *T. harzianum* (155.98 q/ha) and Nettle leaf extract+ *T. viride* (148.39 q/ha) indicating an increase of 51.02, 46.51 and 39.39 per cent, respectively.

Table 3: Efficacy of bio-agents and plant extracts against *Alternaria* leaf spot of brinjal under field conditions

	Treatment Details	Disease Incidence (%)	Disease Intensity (%)	Fruit Yield q/ha
T1	Control	65.51* (7.97)	34.51 (5.95)	106.46
T2	Seed treatment 10^8conidia/ml with *T. harzianum* @ 10^8conidia/ml	51.73 (7.19)	21.72 (4.45)	138.23
T3	Seed treatment with *T. viride* 10^8conidia/ml	58.57 (7.65)	25.54 (4.45)	124.82
T4	Foliar spray with garlic clove extract @10%	32.96 (6. 61)	14.21 (3.89)	134.42
T5	Seed treatment with *T. harzianum* @ 10^8conidia/ml +Foliar spray with garlic clove extract @10%	19.13 (7.73)	9.02 (3.16)	197.80
T6	Seed treatment with *T. viride* @ 10^8conidia/ml + Foliar spray with garlic clove extract @10%	23.92 (5.81)	12.43 (3.60)	179.20
T7	Foliar spray with Datura leaf extract @10%	37.89 (7.13)	21.31 (4.72)	126.04
T8	Seed treatment with*T. harzianum* @ 10^8conidia/ml + Foliar spray with 10% Datura leaf extract @10%	19.23 (6.51)	13.42 (4.77)	166.50
T9	Seed treatment with *T. viride* @ 10^8conidia/ml + Foliar spray with Datura leaf extract @10%	29.79 (7.82)	16.72 (5.15)	160.78
T10	Foliar spray with Nettle leaf extract @10%	40.38 (6.11)	23.31 (4.93)	120.82
T11	Seed treatment with *T. harzianum* @ 10^8conidia/ml + Foliar spray with 10% Nettle leaf extract @10%	24.21 (6.81)	17.42 (3.79)	155.98
T12	Seed treatment with *T. viride* @ 10^8conidia/ml + Foliar spray with Nettle leaf extract @10%	29.77 (6. 60)	18.89 (4.20)	148.39
	C.D. ($p \leq 0.05$)	0.321	0.188	8.13

* Figures in parenthesis are square root transformed values.

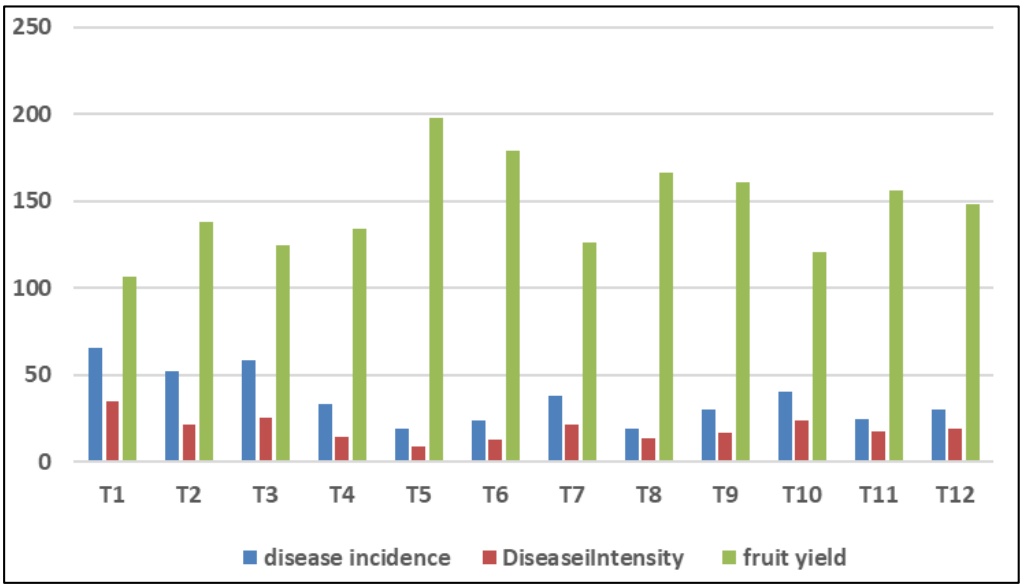

Fig 1: Effect of treatments on disease incidence, intensity *Alternaria* leaf spot of brinjal and yield.

4. Discussion

Alternaria leaf spot infected leaves of brinjal was found to produce typical symptoms, such as small, circular, black-brown concentric rings and necrotic spots and later on turned dark-brown to black-brown in colour. The spots were light brown in colour under the favourable condition and later on turned dark-brown to black-brown in colour. Severely affected leaves drop off. This complies with the symptom reported by Rangaswami and Mahadevan (2010) [21], Raja *et al.* (2006) [19] and Nasehi *et al*. (2012) [14].

The morphological characters of the fungus, studies on PDA media, revealed that the mycelium was septate, branched, dark olive to black measuring 2.17-4.48 µm in diameter, conidiophores were dark brown in colour, septate and measured about 34-93 x 3.4-9.0 µm; conidia were ovoid to obclavate, arranged in long chains of 3-12 conidia measured about 21.82-95.98x8.00-17.00 µm. The morphological characters of the pathogen are same as described by Keissler (1912) [10], Mehrotra and Aggarwal (2003) [11] and Woudenberg *et al.*, (2013) [27]. The identity of fungus was further confirmed by ITCC, Division of Plant Pathology IARI, New Delhi and pathogen was confirmed as *A. alternata* (Fr.) Keissler (Indian Type Culture Collection, No. 10, 2381.16).

Four biocontrol agents *viz*., *T. harzianum*, *T. viride*, *P. fluorescens* and *B. subtillis* were screened *in vitro* against *A. alternata* by dual culture technique. Data on per cent growth inhibition revealed that *T. harzianum* was superior to all other test isolates by exhibiting the maximum mycelial growth inhibition of 83.73 per cent. Among the biocontrol agents,

Bacillus subtilis was least effective and inhibited 51.21 per cent mycelial growth of *A. alternata*. The antagonistic activity may be due to undeniably its mode of action like competition, antibiosis and mycoparasitim. The results are in harmony with earlier workers viz., Ghosh *et al*. (2002) [7] and Akbari and Parakhia (2007) [1], who observed strong antagonism of *T. viride* on *A. alternata.*

Among Plant extract of garlic, datura, walnut, mayweed, nettle, onion, mint and artimesia were tested *in vitro* against the growth of *A. alternata*. Garlic clove extract (80.22%) was found to be most effective in inhibiting the growth of *A. alternata* followed by others. Mayweed leaf extract was least effective in inhibiting growth (23.89%) of *A. alternata*. Those plant extracts found effective *in vitro* were also tested as foliar spray under field conditions.

The most effective, plant extracts and bio-agent evaluated *in vitro* were further evaluated under field conditions against *Alternaria* leaf spot which revealed that seed treatment with bio-agents and foliar sprays with plant extract proved to be significantly superior over their individual treatments in suppressing the disease severity. Seed treatment with *Trichoderma harzianum* followed by two foliar sprays of Garlic clove extract @ 10 per cent at 45 and 55 days after transplanting was found to be the best and highly effective in reducing the disease severity (9.02%), and gave maximum fruit yield of 197.80 q/ha compared to control, wherein it recorded the highest disease intensity (34.51%) and the lowest fruit yield of (106.46 q/ha). These results are in agreement with the result of Jadeja and Pipliya (2008) [8], Panchal and Patil (2009) [16], Vihol *et al.* (2009) [24], Gangopadhay *et al.* (2010) [6] and Bochalya *et al*. (2012) [4].

The combined treatments, viz., Bio-agents + Plant extract were significantly superior over individual treatments. The results thus explain the viability of a package integrating, bio-agents and plant extracts for the management of *Alternaria* leaf spot of brinjal.

The conclusion drawn from the present investigations is presented as follows:

- The disease incitant was identified as *Alternaria alternata* (Fr.) Keissler
- The bio-control agent *Trichoderma harzianum* proved effective against the test pathogen.
- The plant extracts such as Garlic clove extract, Datura leaf and Nettle leaf extract were effective against test pathogen.
- The eco-friendly disease management capsule developed for the management of *Alternaria* leaf spot of brinjal is seed treatment with *Trichoderma harzianum* followed by foliar spray with Garlic clove extract @ 10 per cent.
- The combined treatments, viz., Bio-agents + Plant extract were significantly superior over individual treatments. The results thus explain the viability of a package integrating, bio-agents and plant extracts for the management of *Alternaria* leaf spot of brinjal.

5. References

1. Akbari LF, Parakhia AM. Management of *Alternaria alternata* causing blight of sesame with fungicides. Journal of Mycology and Plant Pathology 2007;37(3):426-430.
2. Anonymous. Vegetables Package of Practices manual vol. ii. Directorate of Extension education, SKUAST-Kashmir 2011.
3. Balai P, Ahir R, Yadav S. Varietal screening of brinjal genotypes against leaf spot of disease caused by *Alternaria alternata*. Environment and Ecology 2010;31:1276-1278.
4. Bochalya S, Shkhawat S, Singh R, Chohan PK. Studies on age of fruit, age of inoculum and inoculum load on development of *Alternaria* fruit rot of brinjal caused by *Alternaria alternata*. Annals of Agri Bio Research 2012;18:388-390.
5. Dennis C, Webster J. Antagonistic properties of species-groups of Trichoderma: III. Hyphal interaction. Transactions of the British Mycological Society 1971;57(3):363-369.
6. Gangopadhay S, Bhakar B, Godara S. Effect of botanicals on cumin blight caused by *Alternaria burnssi*. Journal of Mycology and Plant Pathology 2010;40(4):570-574.
7. Ghosh C, Pawar N, Kshirsagar C, Jadhav A. Studies on management of leaf spot caused by *Alternaria alternata* on gerbera. Journal of Maharashtra Agricultural Universities 2002;27:163-167.
8. Jadeja K, Pipliya B. *In vitro* evaluation of Plant extracts and bioagents against *Alternaria burnsii* Uppal, Patel & Kamat causing blight of cumin (*Cuminum cyminum* L.) J. Spices and Aromatic Crops 2008;17:26-28.
9. Johnston A, Booth C. Plant pathologist's pocketbook, 2nd edn. Commonwealth Agricultural Bureaux. International Mycological Institute, Kew, England 1983.
10. Keissler K. Zur Kenntnis der Pilzflora Krains. Beihefte Zum Botanischen Zentralblatt 1912;29:395- 440.
11. Mehrotra R, Aggarwal A. Leaf spot and leaf blight, In: *Plant Pathology*, 2nd edition, Tata Mcgraw- Hill Publishing Co. Ltd 2003, Pp. 505-510.
12. Miller D, Guynn S, Wigley T, Guynn D. Herbicides and forest biodiversity—what do we know and where do we go from here? Wildlife Society Bulletin 2004;32:1085-1092.
13. Mishra M, Tewari S. Ethanolic extract toxicity of three botanicals against five fungal pathogens of rice. National Academy of Science 1990;13:409-412.
14. Nasehi A, Kadir J, Abidin M, Wong M, Mahmodi F. First report of *Alternaria tenuissima* causing leaf spot on eggplant in Malaysia. Plant Disease 2012;96:1226-1226.
15. Nigam S, Misra G, Sharma A. Neem: A promising Natural insecticide, Applications in Plant Sciences 1994;14:35-46.
16. Panchal D, Patil R. Eco-friendly management of fruit rots of tomato caused by *Alternaria Alternata*. Journal of Mycology and Plant Pathology 2009;39:66-69.
17. Pandey A, Pandey B. Fungal diseases of brinjal in Bareilly region. London, USA 2001, pp.333-361.
18. Raina ZK, Rather RA, Bhat SA, Wani TA, Bhat NA, Dorjey S, *et al*. Severity of *Alternaria* Leaf Spot of Brinjal causedby *Alternaria alternata* in Kashmir, India Int. J. Curr. Microbiol. App. Sci 2018;7(5):322-328
19. Raja P, Reddy A, Allam U. First report of *Alternaria tenuissima* causing leaf spot and fruit rot on eggplant (*Solanum melongena*) in India. Plant pathology 2006;55(4):579-579.
20. Rajan S, Markose BL. Propagation of Horticultural Crops. Horticulture Science Series 2002;6:94.
21. Rangaswami G, Mahadevan A. Diseases of Cereals", in Diseases of Crop Plants in India, 4th edition PHI Learning Pvt. Ltd. New Delhi (India) 2010, pp.160-162.
22. Schmitz H. Poisoned food technique. Industry of Engineering Chemical Advances in Plant Sciences 1930;14:99-104.

23. Singh P, Srivastava D. Phytochemical Screening and *In Vitro* Antifungal Investigation of *Parthenium Hysterophorus* extracts against *A. alternata*. International Research Journal of Pharmacy 2013;4:190-193.
24. Vihol J, Patel K, Jaiman R, Patel N. Efficacy of plant extracts, biological agents and fungicides against *Alternaria* blight of cumin. Journal of Myocolgy and Plant Pathology 2009;39(3):516-519.
25. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 1927;15(4051):850.
26. Wally F, Taylor A, Lupwayi N. Herbicide residues & effects on nitrogen fixation in pulse crops. Farm Tech 2006, 52-55.
27. Woudenberg J, Groenewald J, Binder M, Crous P. *Alternaria* redefined. Studies in Mycology 2013;75:171-212.