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Epidemiological studies on the Incidence and Distribution of Leaf Spot Disease in Ashwagandha (*Withania somnifera*) Caused by *Alternaria alternata*

Sudhanshu Vats[1], Ram Suman Mishra[2], Abhishek Katiyar[3]

[1]M.Sc., Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India.

[2] Associate Professor, Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India.

[3]Ph.D. Research Scholar, Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India.

Corresponding author Email: sudhanshuvats27@gmail.com

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ABSTRACT

Medicinal and aromatic plant *Withania somnifera* (Ashwagandha), is integral to Indian traditional medicine and the herbal pharmaceutical industry, owing to their rich content of bioactive compounds like alkaloids, glycosides, flavonoids. However, their commercial cultivation is severely impacted by fungal diseases, particularly foliar infections, which reduce yield, compromise quality, and alter the efficacy of pharmacologically important metabolites. Ashwagandha showed early disease symptoms in the 43rd SMW, with severity peaking at 38.77% in the 13th SMW due to rainfall and sustained humidity. In Ashwagandha, T9 achieved over 81% control for both metrics at early stages.

Introduction -

Epidemiology has made significant contributions to plant pathology by elucidating the general principles underlying the development of disease epidemics and provided analytical prospective of theoretical and empirical understanding of the dynamics of disease epidemics in time and space (Hughes and Burnett, 2017), predictions of disease outbreaks or the need for disease control in real-time basis, and tactical and strategic solutions to disease problems (Ojiambo, 2017). Medicinal and aromatic plants constitute a major segment of the flora, which provides raw materials for use in the pharmaceuticals, cosmetics and drug

industries (Jeelani- *et. al*.2018). The global market for plant-based drugs is estimated at $100billion, projected to reach $5 trillion by 2050, it is because of about 80% of the population relying on herbal medicine (Andrea, 2011). Medicinal and aromatic plants are attacked by number of fungal, bacterial, viral and nematode pathogens which affect its growth as well as alter its therapeutics properties. Biotic pathogens are spread rapidly throughout the crop under wet and humid environmental conditions. Among the several diseases of Ashwagandha the leaf spot of is one of the devastating diseases.

Materials and Methods:

The research experiment entitled **"Epidemiological studies on disease occurrence in medicinal and aromatic plants especial reference to Basil , Kalmegh, Ashwagandha and Opium poppy"** was carried out at Medicinal and Aromatic Plant farm, Main Horticulture Experiment Station, Department of Plant pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.) India during, Kharif and *Rabi* 2024-25.

Collection of disease samples

The diseased samples were collected from the experimental site. Infected leaves were gathered in sterilized polybags. The infected parts showing the typical symptom of the associated was brought to the laboratory for isolation of the pathogens on PDA media or distilled water. The pure culture was maintained on PDA slant and distilled water for further studies.

Visual examination

On the basis of visual observations of plant, the typical symptoms of diseases on Kalmegh (leaf spot), Ashwagandha (leaf spot), Basil (downy mildew) and Opium (downy mildew) were recorded and noted in the field.

Disease incidence and severity

Weekly data of disease incidence and disease severity of leaf spot of Ashwagandhan was collected from 1st week of December to 2nd week of March in both the year.. The weekly disease incidence and severity data were recorded using 0 - 5 point scale after the appearance of downy mildew.

Downy mildew disease rating scale (Natti *et al.*, 1967)

Score/Grade	Description of disease symptoms
0	Cotyledons/ leaves free from infection
1	Small creamy white to light brown spots on cotyledons/leaves covering 1-10% area
2	Small creamy white to light brown spots with cottony growth on lower surface covering 10.1-25% cotyledons/leaves area
3	Creamy white to light brown spots with cottony growth covering 25.1-50% cotyledons/leaves area
4	Creamy white to light brown spots with cottony growth covering 50.1-75% cotyledons/leaves area
5	Creamy white to light brown spots with cottony growth covering >75% cotyledons/leaves area

The percent disease incidence was calculated using formula given by Ginting and Maryono, 2009.

$$\text{Percent Disease Incidence} = \frac{\text{Total no.of infected plant}}{\text{Total no.of plant planted}} \times 100$$

Disease severity was calculated on weekly intervals with the following formula given by Shutong *et. al*., 2007.

$$\text{Percent Disease Severity} = \frac{Sum\ of\ all\ numerical\ rating}{Number\ of\ plants\ scored \times maximum\ score\ on\ scale} \times 100$$

Experimental Details.

Season	**Kharif**
Crop	**Ashwagandha**
Design	**RBD**
Replications	**3**
Treatment	**10**
Plot Size	**3×2.40 m^2**
Spacing	**50×30cm**

Table.1 Treatment of Integrated Disease Management for downy mildew (Ashwgandha)

Treatment	
T_1	Seed treatment with *Pseudomonas fluorescence* (5g/kg) + *Trichoderma harzianum*(10g/kg) each + Soil application of FYM enriched with *Trichoderma* + *Pseudomonas* (2.5kg formulation in 400kg of FYM) + first foliar spray of jeevamrit @ 10% with the onset of disease followed by 2nd and 3rd at 15days interval.
T_2	Seed treatment with *Pseudomonas fluorescence* (5g/kg) + *Trichoderma harzianum*(10g/kg) each + Soil application of FYM enriched with *Trichoderma* + *Pseudomonas* (2.5kg formulation in 400kg of FYM) + first foliar spray of *Pseudomonas fluorescence* + *Trichoderma harzianum* each @ 1% onset of disease followed by two sprays at 15 days interval.
T_3	Seed treatment with *Pseudomonas fluorescence* (5g/kg) + *Trichoderma harzianum*(10g/kg) each + Soil application of FYM enriched with *Trichoderma* + *Pseudomonas* (2.5kg formulation in 400kg of FYM) + first foliar spray of Azadiractin 1500ppm @ 2.5 ml/liter onset of disease followed by two sprays at 15 days interval.
T_4	Seed treatment with *Pseudomonas fluorescence* (5g/kg) + *Trichoderma harzianum*(10g/kg) each + Soil application of FYM enriched with *Trichoderma* + *Pseudomonas* (2.5kg formulation in 400kg of FYM) + first foliar spray of Chitosan @0.1% with the onset of disease followed by two sprays at 15days interval.
T_5	Seed treatment with jeevamrit @10ml/l + Soil application of FYM enriched with *Trichoderma* + *Pseudomonas* formulation each (2.5kg formulation in 400kg of FYM) + first foliar spray of Azadiractin 1500ppm @ 2.5 ml/liter onset of disease followed by two sprays at 15 days interval.

T_6	Seed treatment with jeevamrit @10ml/l + Soil application of FYM enriched with *Trichoderma* + *Pseudomonas* formulation each (2.5kg formulation in 400kg of FYM) + first foliar spray of jeevamrit @10% with the onset of disease followed by 2nd and 3rd at 15 days interval.
T_7	Seed treatment with jeevamrit @10ml/l + Soil application of FYM enriched with *Trichoderma* + *Pseudomonas* formulation each (2.5kg formulation in 400kg of FYM) + first foliar spray of Chitosan @0.1% with the onset of disease followed by two sprays at 15 days interval.
T_8	Seed Treatment with chitosan 0.1% + first foliar spray of Chitosan @ 0.1% with the onset of disease followed by two sprays at 15days interval.
T_9	Chemical control (50% Tebuconazole and 25% Trifloxystrobin)
T_{10}	**Control**

Percent Disease Incidence

$$\text{Percent Disease Incidence} = \frac{\text{Total no.of infected plant}}{\text{Total no.of rhizome planted}} \times 100$$

Percent Disease Severity

$$\text{Percent Disease Severity} = \frac{\text{Sum of all numerical rating}}{\text{Number of plants scored} \times \text{maximum score on scale}} \times 100$$

Results and Discussion:

Effect of Integrated disease management on leaf spot of Aswhagandha

Leaf spot of Aswhagandha

Experimental data revealed that all the treatments were significantly minimized the Leaf spot disease of aswhagandha (table -3). The highest disease incidence was recorded in T_{10} (47.29%) followed by T_6 (36.39%) ,T_2 (33.34 %) and T_1 (30.49%) and lowest in T_9 (8.73%) followed by T_8 (10.11%), T_5 (10.89%) and T_4 (14.37%) at 30 days of transplanting(DAT). In case of 45 DAT, maximum disease incidence was recorded in T_{10} (49.93%), followed by T_6 (40.77%) ,T_2 (39.85 %) and T_1 (37.55%) and lowest in T_9 (11.88%) followed by T_8 (18.89 %), T_5 (20.22%) and T_4 (21.43%) . The highest disease incidence was recorded in T_{10} (55.20%) followed by T_6 (45.44%) ,T_2 (43.99 %) and T_1 (43.63%) and lowest in T_9 (18.22%) followed by T_8 (23.28%), T_5 (27.44%) and T_4 (28.48%) at 60 days of transplanting (DAT). In case of 75 DAT, maximum disease incidence was recorded in T_{10} (59.40%), followed by T_6 (53.88%) ,T_2 (51.44 %) and T_1 (49.75%) and lowest in T_9 (22.87%) followed by T8(27.38 %), T_5 (31.10%) and T_4 (31.28%) .

Where as in disease severity it was found maximum in T_{10} (23.05%) followed by T_6 (15.05%), T_2 (14.08%) and T_1 (13.15%) and minimum in T_9 (4.22%) followed by T_8 (5.89%), T_5 (6.19%) and T_4 (7.21%) at 30 days of transplanting (DAT). In case of 45 DAT, the highest disease severity T_{10} (28.44%) followed by T_6 (20.75%), T_2 (20.35%) and T_1 (18.88%) and lowest in T_9 (8.06%) followed by T_8 (10.10%), T_5 (11.87%) and T_4 (12.77%). Disease severity it was found maximum in T_{10} (33.77%) followed by T_6 27.60%), T_2 (26.49%) and T_1 (25.08%) and minimum in T9 (11.55%) followed by T8 (14.66%), T5 (16.22%) and T4 (19.09%) at 60 days of transplanting (DAT). In case of 75 DAT, the highest disease severity T10 (38.77%) followed by T_6 (32.09%), T_2 (30.00%) and T_1 (28.99%) and lowest in T_9 (16.38%) followed by T8 (18.83%), T5 (21.57%) and T4 (24.80%). Sarkar (2021) reported that the highest disease (30.61%) was in control treatment and application of Carbendazim recorded the lowest percent disease incidence (14.75%) in comparison to control (74.99 %). These results are similar to previous researcher like as Mishra (2021) and Kathal (2023).

Table 2: Effect of Integrated disease management on leaf spot of Aswhagandha

Treatments	Disease appearance Days after transplanting	Percent Disease Incidence Days of transplanting (DAT)				Percent Disease Severity Days of transplanting (DAT)			
		30	45	60	75	30	45	60	75
T_1	30	30.49 (33.50)	37.55 (33.77)	43.63 (41.32)	49.75 (44.84)	13.15 (21.25)	18.88 (25.73)	25.08 (30.04)	28.99 (32.56)
T_2	30	33.34 (35.25)	39.85 (39.12)	43.99 (41.53)	51.44 (45.80)	14.08 (22.02)	20.35 (26.80)	26.49 (30.95)	30.00 (33.19)
T_3	31	26.56 (31.00)	31.20 (33.94)	38.67 (38.43)	45.68 (42.50)	12.70 (20.86)	17.42 (24.65)	21.25 (27.43)	25.83 (30.53)
T_4	32	14.37 (22.26)	21.43 (27.56)	28.48 (32.24)	31.28 (33.99)	7.21 (15.56)	12.77 (20.96)	19.09 (25.89)	24.80 (29.85)
T_5	32	10.89 (19.25)	20.22 (26.70)	27.44 (31.57)	31.10 (33.87)	6.19 (14.39)	11.87 (20.14)	16.22 (23.51)	21.57 (27.65)
T_6	30	36.39 (37.08)	40.77 (39.63)	45.44 (42.36)	53.88 (47.20)	15.05 (22.81)	20.75 (27.08)	27.60 (31.68)	32.09 (34.48)
T_7	30	29.82 (33.08)	35.29 (36.43)	41.01 (39.80)	46.87 (3.19)	13.02 (21.14)	17.72 (24.87)	23.05 (28.67)	28.43 (32.20)
T_8	32	10.11 (18.52)	18.89 (25.75)	23.28 (28.83)	27.38 (31.53)	5.89 (14.02)	10.10 (18.51)	14.66 (22.50)	18.83 (25.69)
T_9	34	8.73 (17.14)	11.88 (20.15)	18.22 (25.25)	22.87 (28.55)	4.22 (11.83)	8.06 (16.46)	11.55 (19.84)	16.38 (23.85)
T_{10}	30	47.29 (43.42)	49.93 (44.94)	55.20 (47.96)	59.40 (50.39)	23.05 (28.68)	28.44 (32.21)	33.77 (35.51)	38.77 (38.49)
C.D. (P=0.5%)		**1.858**	**1.379**	**1.588**	**1.720**	**1.022**	**1.477**	**1.335**	**1.837**
SE(m) ±		**0.620**	**0.460**	**0.530**	**0.574**	**0.341**	**0.493**	**0.446**	**0.614**
C.V. %		**4.333**	**2.598**	**2.514**	**2.371**	**5.158**	**5.136**	**3.535**	**4.000**

(Data in the parenthesis is transformed values)

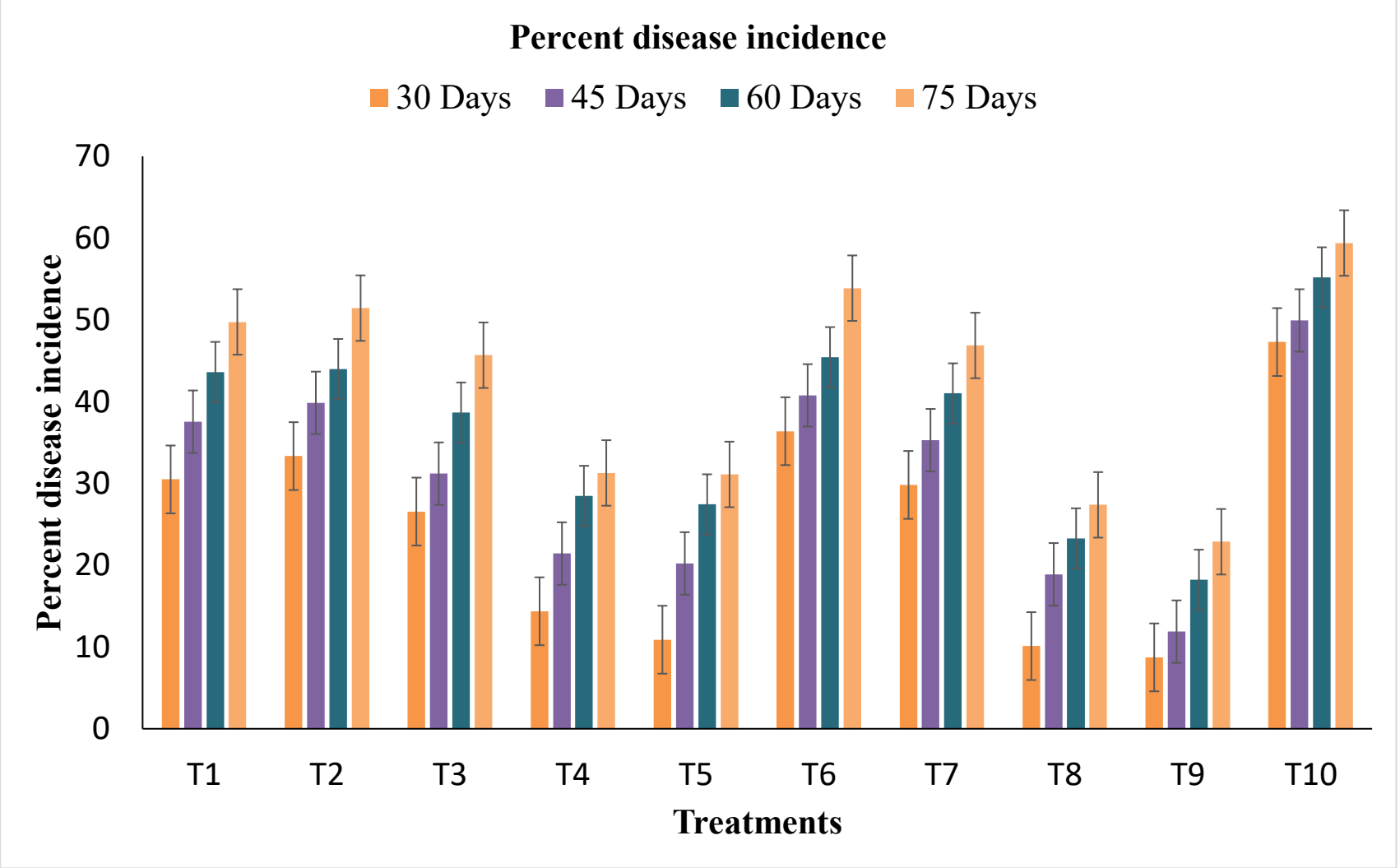

Fig 1: Effect of Integrated disease management on leaf spot of Ashwgandha

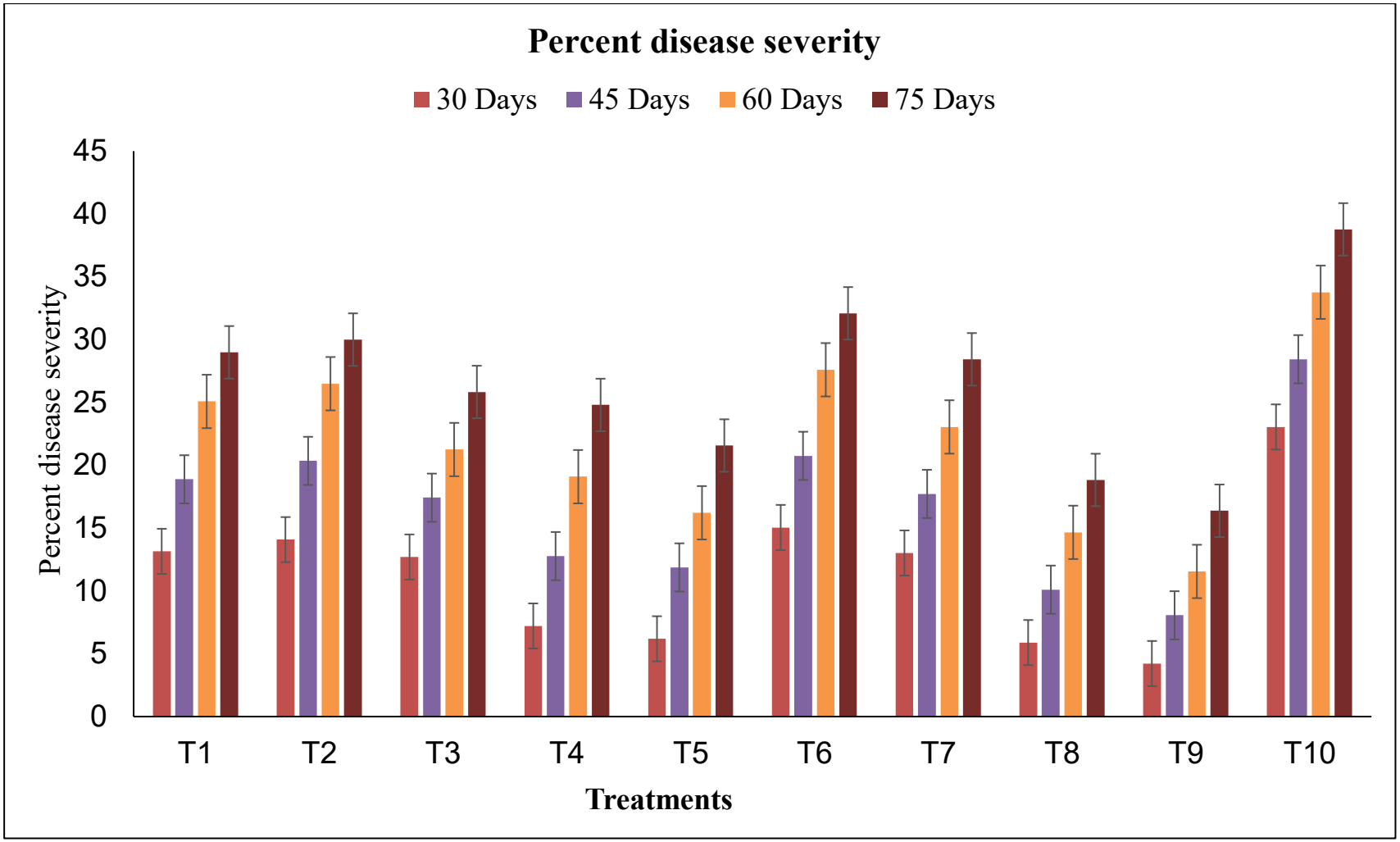

Fig 2: Effect of Integrated disease management onleaf spot of Ashwgandha

Summary and Conclusion

Ashwagandha (*Withania somnifera*) – Alternaria Leaf Spot

1. Leaf spot of Ashwagandha was found to be affected by *Alternaria alternata*, First symptoms recorded at 34 DAS in T_9 as large, zonate, concentric necrotic lesions with brown margins. The lesions appeared mostly on the older leaves but later covered the entire
2. Microscopic observation showed the presence of muriform, beaked conidia with longitudinal and transverse septa, confirming the identity of *A. alternata*.
3. Lowest disease incidence was observed in T_9 8.73 %, 11.88%, 18.22% and 22.87% followed by T_8 10.11%, 18.89% , 23.28% and 27.38%, T_5 10.89%, 20.22%, 27.44% and 31.10% at 30,45,60 and 75 DAS respectively .
4. Minimum disease severity was recorded in T_9 4.22%, 8.06% ,11.55%, and 16.38% followed by T_8 5.89%, 10.10 %, 14.66% and 18.83%, T_5 6.19%, 11.87%, 16.22% and 21.57% at 30,45,60 and 75 DAS respectively .
5. Maximum per cent disease incidence and severity control was observed in T_9 61.48, 57.78 followed by T_4 53.91, 51.47 at 75DAT.
6. Weekly increase rate of leaf spot in Ashwagandha (0.46) was recorded at 50th meteorological week when average maximum and minimum temperature was 23.1°C and 4.0°C, with relative humidity 63.4%.
7. The leaf spot disease reached its peak severity 38.77%. in the 13th SMW when maximum and minimum temperatures were 35.5°C and 14.8°C, 63.6 mm of rainfall,
8. Total evaporation exhibited the strongest positive correlation with disease severity
   ($r = 0.5826$), with an R^2 value of 0.1666 and regression equation $y = 0.4112x + 21.553$.

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