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"Evaluation Of Specific Efficacy Of Fungicides, Bio Agents And Botanicals Against Leaf Spot Caused By *Alternaria Alternata* In Green Gram (*Vigna Radiata L.*): An Integrated Disease Management Approach"

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Abstract
The present study investigated in the *kharif* 2022 and 2023 in RBD for check the efficacy of Different methods for managing leaf spot disease caused by *Alternaria alternata*.. Evaluated the impact of fungicides, bio agents and botanicals treatments at three distinct stages of crop growth: 30 days after sowing (DAS), 60 DAS and 90 DAS, providing a comprehensive understanding of their effectiveness over time. In research trails minimum percent disease incidence was recorded in treatment T_4 - Foliar spray of *T. viride* @ 10^8 conidia/ml+Foliar spray with Neem oil @ 0.5%+ Foliar spray@ 0.1% Propiconazole 25% EC with pooled (*kharif* 2022 and 2023) 26.015, 33.55 and 45.00 at 30.60 and 90 DAS, respectively. Whereas treatment T_4 - Foliar spray of *T. viride* @ 10^8 conidia/ml+Foliar spray with Neem oil @ 0.5%+ Foliar spray@ 0.1% Propiconazole 25% EC showed maximum seed yield 653.12 (kg/ha). The results highlight the importance of adopting a stage-specific approach to disease management, as the impact of *Alternaria alternate* and the effectiveness of treatments can vary significantly throughout the crop growth cycle. This research provides valuable insights into optimizing disease management strategies for *A. alternata* in *Kharif* crops, emphasizing the need for a holistic approach that integrates various control measures.

Keywords:- Alternaria alternata, Trichoderma viride, Neem oil, Propiconazole, Foliar spray, Disease incidence, Kharif crops, Seed yield

Introduction:-
Green gram (Vigna radiata L.), also known as mung bean, is an important legume crop. extensively cultivated in Rajasthan. Key districts such as Jodhpur, Nagour, Ganganagar, Ajmer, Bikaner and Jaipur are major producers.
Green gram seeds are nutritionally rich, especially in protein and micronutrients such as iron, calcium, magnesium and potassium. Known as "poor man's meat," green gram provides 24 g of protein per 100 g, surpassing common beans, which offer 21 g. Additionally, the plant's stalks and pods serve as valuable leguminous fodder for livestock, containing 10-15% raw protein, 20-26% raw fiber, 2-2.5% ether extract, 40-49% nitrogen-free extract, and 11-15% mineral content. when dried. Green gram also contributes significantly to soil health by fixing nitrogen, increasing soil nitrogen levels by up to 30-40 kg per hectare post-harvest (Taylor & Francis, 2015).
Rajasthan plays a crucial role in India's green gram production despite its arid and semi-arid conditions. Key growing districts include Jodhpur, Bikaner, Barmer, Jaisalmer, Nagaur, Hanumangarh, Churu, and Sri Ganganagar (Sitaram et al., 2014). India, as the leading global producer, cultivates green gram on approximately 46.07 lakh hectares, yielding 24.48 lakh tonnes with a productivity of 531 kg/ha. In the 2020-21 period, mung beans accounted for 10% of India's total pulse production during both *Kharif* and *Rabi* seasons, with Rajasthan being the top producer (DES, 2020-21).
However, green gram cultivation is severely hampered by various diseases, with leaf spot caused by fungal pathogens being a primary concern. These diseases not only diminish crop yield but also negatively influence the quality of the produce, thus undermining the economic sustainability of farming practices.
Effective management strategies are essential to reduce the impact of leaf spot diseases on green gram.. Integrated Disease Management (IDM) approaches that combine cultural practices, chemical treatments and biological control agents have been explored to achieve sustainable disease control. In this context, the present study evaluates seven different management treatments under a randomized block design to assess their efficacy in controlling leaf spot disease and enhancing seed yield.

Materials and Methods
During the *Kharif* seasons of 2022 and 2023, samples showing disease symptoms were collected from green gram fields located in various districts of Rajasthan. The diseased aerial portions of the plants were meticulously excised and placed in polyethylene bags, each properly labeled, and then transported to the laboratory.. Upon arrival, all samples underwent a rigorous isolation procedure to extract the pathogen. The surfaces of the affected aerial parts were carefully cleaned by rinsing them under flowing tap water to eliminate any clinging dirt and soil.Small sections of these samples were then excised using sterilized scalpels, washed again in sterilized water and subjected to surface sterilization. The process

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involved immersing the sterilized sections in a 0.1% mercuric chloride ($HgCl_2$) solution for two minutes, followed by three washes in sterilized distilled water. Afterward, these treated sections were placed on sterilized potato dextrose agar (PDA) medium in Petri dishes. The plates were subsequently incubated at 26±1°C in a BOD incubator. After 3-4 days, mycelia growth around the periphery was noted. Sections exhibiting this growth were meticulously isolated using a sterilized inoculation needle and transferred onto fresh, sterilized PDA plates for sub-culturing under strictly aseptic conditions.

Bio-control agents were isolated from the rhizosphere soil of both healthy and diseased green gram plants using specific techniques aimed at isolating fungal bio-control agents and antagonistic microorganisms while maintaining anonymity.

For the isolation of antagonistic bacteria, selective King's B media (King *et al.*, 1954) was utilized. A stock soil suspension was prepared by combining 10 g of soil with 90 ml of sterile distilled water in an Erlenmeyer flask and incubating with gentle agitation for 3 to 6 minutes. Serial dilutions up to 10^8 were performed from this stock suspension. A 0.2 ml aliquot from the selected dilution was then evenly distributed on the surface of Petri dishes containing the growth media using a sterile glass spreader.The inoculated plates were then incubated at 26 ± 20°C for 24 hours and the resulting bacterial colonies were sub cultured onto King's B media for identification and subsequent laboratory use.

The efficacy of fungicides, biological agents, and botanical extracts, previously deemed promising under laboratory conditions, was assessed both in isolation and in synergistic combinations.. The field trial was conducted using a randomized block design (RBD) with three replicates. Green gram seeds (SKAU-M-86) were sown in plots assigned to each treatment. Inoculations were performed using the most virulent isolate (NAG Aa-1) identified from pathogenicity studies of *A. alternata*. An inoculated control

Without application of fungicides, botanicals or bio agents was maintained for comparison. Disease severity was assessed at 15-day intervals until seed maturity using a standard disease rating scale (0-5 score).

The percentage disease index (PDI) and percentage efficacy of disease control (PEDC) were determined using the methodologies outlined by Chester (1959) and Wheeler (1969).

$$\text{Percentage Disease Index (PDI)} = \frac{\text{Sum of all individual disease rating}}{\text{Total number of plant species X the highest achievable rating}} \times 100$$

$$\text{PEDC} = \frac{\text{PDI in control} - \text{PDI treatment}}{\text{PDI in Control}} \times 100$$

Treatment details:

T_1 - Treatment with promising fungicide of Propiconazole 25% EC found best effective in *in vitro* Foliar spray@ 0.1%.

T_2 - Foliar spray of *T. viride* @ 10^8 conidia/ml+Foliar spray with Neem oil @ 0.5%+ Foliar spray@ 0.1% Propiconazole 25% EC

T_3 - Foliar spray of Propiconazole 25% EC @ 0.1% + Spray of Neem oil @ 0.5%

T_4 - Foliar spray of Neem oil @ 0.5%

T_5 - Foliar spray of *T. viride* @ 10^8 conidia/ml + Foliar spray with Neem oil @ 0.5%

T_6 - Foliar spray with *T. viride* @ 10^8 conidia/ml

T_7 - Control

Results

To assess sustainable management strategies for *Alternaria alternata* in green gram, field trials were executed across two consecutive Kharif seasons (2022 and 2023) at the research farm of Vivekananda Global University, Jaipur. The experimental design utilized was a Randomized Block Design (RBD) with three replications. Thirty-day-old plants were inoculated with a spore suspension containing 1 x 10^3 conidia ml^-1 of the most virulent isolate, NAG Aa-1, identified from in vitro studies of *A. alternata*. The efficacy of fungicide Propiconazole 25% EC, botanical Neem oil (20%) and bioagent *T. viride*, identified as most effective *in vitro*, was evaluated through foliar spray applications outlined in the Materials and Methods section.

Disease severity was evaluated using a standardized scale ranging from 0 to 5, with the quantity of plants assigned each severity score documented at 30, 60, and 90 days after inoculation. The Percent Disease Index (PDI) and Percent Efficacy of Disease Control (PEDC) were subsequently computed based on these data points.Grain yield was measured, and the percent yield increase over the control was determined as detailed in the Materials and Methods. Table 1,2,3 and 4 presents the results of this study.

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Table 1: Efficacy of various treatments of Antifungal agents, biocontrol microorganisms, and plant-derived compounds against Alternaria leaf spot *in green gram* at 30 DAS during *Kharif* 2022 and 2023

S.No.	Treatments	Percent disease incidence after 30 DAS*			Percent efficacy of Disease control (PEDC)**		
		Kharif 2022	*Kharif* 2023	Pooled	*Kharif* 2022	*Kharif* 2023	Pooled
1.	T_1	17.57	15.96	16.765	41.13 (45.36)	43.10 (46.85)	42.11 (44.75)
2.	T_2	11.45	12.6	12.025	61.64 (65.82)	55.08 (53.76)	58.36 (61.83)
3.	T_3	14.72	13.25	13.985	50.68 (55.82)	52.76 (54.69)	51.72 (56.75)
4.	T_4	25.91	26.12	26.015	13.19 (17.60)	6.88 (8.96)	10.03 (12.46)
5.	T_5	18.82	19.15	18.985	36.95 (39.85)	31.72 (32.46)	34.33 (38.94)
6.	T_6	21.30	23.00	22.15	28.64 (32.16)	18.00 (22.85)	23.32 (26.89)
7.	T_7-Control	29.85	28.05	28.95	00.00	00.00	00.00
SEm ±		0.320	0.295	0.220	1.166	0.865	0.622
CD at 5%		1.124	1.651	0.687	3.542	2.722	1.654
C.V		3.12	2.81	3.05	4.838	3.60	4.33

Table 2: Efficacy of various treatments of Antifungal agents, biocontrol microorganisms, and plant-derived compounds against Alternaria leaf spot *in green gram* at 60 DAS during *Kharif* 2022 and 2023

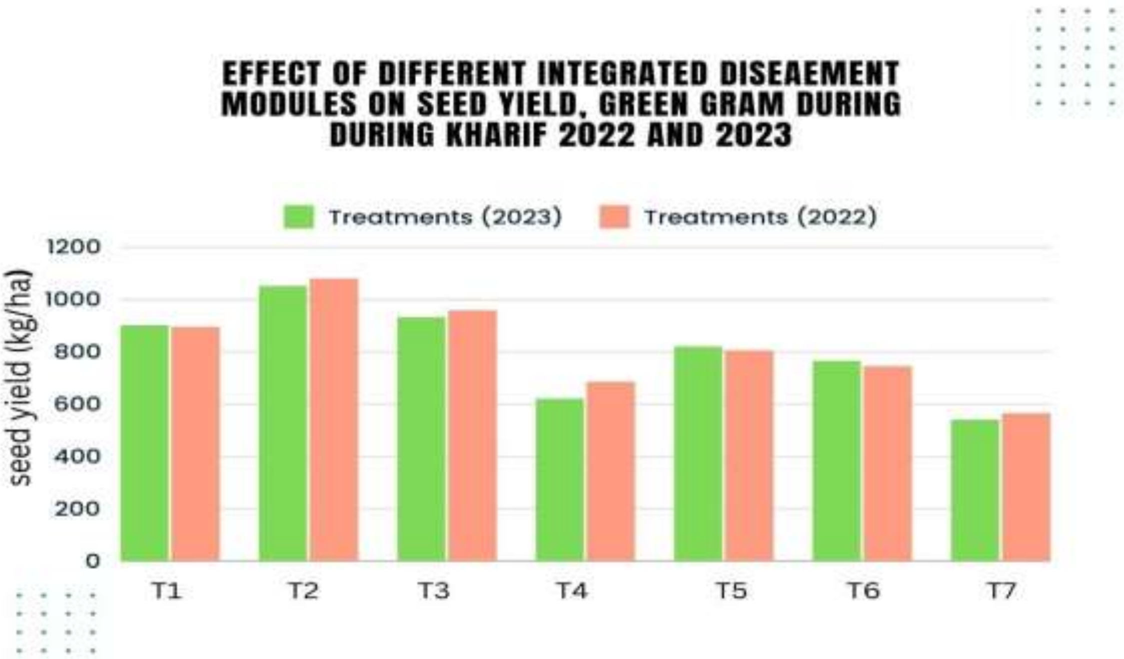
S.No.	Treatments	Percent disease incidence after 60 DAS*			Percent efficacy of Disease control (PEDC)**		
		Kharif 2022	Kharif 2023	Pooled	Kharif 2022	Kharif 2023	Pooled
1.	T_1	23.56	24.00	23.78	41.34 (44.62)	37.18 (39.75)	39.26 (41.30)
2.	T_2	15.70	16.00	15.85	60.91 (63.75)	58.12 (61.85)	59.51 (62.78)
3.	T_3	20.45	19.12	19.78	49.09 (54.85)	49.96 (53.75)	49.52 (54.63)
4.	T_4	34.00	33.10	33.55	15.35 (18.00)	13.37 (15.50)	14.36 (16.85)
5.	T_5	26.98	27.03	27.00	32.83 (34.85)	29.25 (33.30)	31.04 (34.28)
6.	T_6	31.00	30.05	30.52	22.82 (24.68)	21.35 (25.85)	22.08 (28.64)
7.	T_7-Control	40.17	38.21	39.19	00.00	00.00	00.00
SEm ±		0.503	0.455	0.204	0.710	0.738	0.475
CD at 5%		1.511	1.486	0.866	2.373	2.176	1.321
C.V		3.43	3.35	3.21	3.14	2.87	3.13

Table 3: Efficacy of various treatments of Antifungal agents, biocontrol microorganisms, and plant-derived compounds against Alternaria leaf spot *green gram* at 90 DAS during *Kharif* 2022 and 2023

Sl. No.	Treatments	Percent disease incidence after 90 DAS*			Percent efficacy of Disease control (PEDC)**		
		Kharif 2022	*Kharif* 2023	Pooled	Kharif 2022	Kharif 2023	Pooled
1.	T_1	33.75	34.85	34.30	36.01 (41.25)	32.52 (36.56)	34.26 (38.69)
2.	T_2	24.40	23.94	24.17	53.74 (57.12)	53.64 (55.38)	53.69 (57.10)
3.	T_3	29.55	29.90	29.72	43.98 (49.00)	42.11 (46.75)	43.04 (47.63)
4.	T_4	44.95	45.05	45.00	14.78 (16.82)	12.77 (15.35)	13.77 (16.85)
5.	T_5	37.06	37.51	37.28	29.74 (32.01)	37.69 (38.96)	33.71 (34.85)
6.	T_6	41.45	40.00	40.72	21.42 (24.85)	22.55 (26.61)	21.98 (25.85)
7.	T_7–Control	52.75	51.65	52.20	00.00	00.00	00.00
SEm ±		0.650	0.724	0.418	0.824	0.736	0.481
CD at 5%		2.035	2.204	1.234	2.527	2.228	1.331
C.V		3.20	3.57	3.42	3.43	3.11	3.29

Table 4: Effect of various treatments of different integrated disease management modules on seed yield, 'SKAU-M- 86' during during *Kharif* 2022 and 2023

Sl. no.	Treatments	Seed yield (kg/ha*)		
		Kharif 2022	Kharif 2023	Pooled
1.	T_1	896.30	901.65	898.97
2.	T_2	1080.45	1050.85	1065.65
3.	T_3	958.00	932.90	945.45
4.	T_4	685.85	620.40	653.12
5.	T_5	805.65	820.55	813.10
6.	T_6	745.45	765.60	755.52
7.	T_7 -Control	565.60	540.50	553.05
SEm±		24.406	25.620	15.356
CD at 5%		75.267	78.915	44.712
C.V		5.40	5.57	5.49



Discussion

Treatment T_2 was the most effective against Alternaria alternata, with the lowest disease incidence (12.025%) and the highest efficacy (58.36%). Treatment T_3 also showed good efficacy (51.72%). In contrast, the control (T_7) had the highest disease incidence (28.95%) and no efficacy. Treatments T_4, T_5, and T_6 had varying degrees of lower efficacy. Statistical analysis confirmed significant differences between treatments, with T_2 consistently providing the best disease control. (Table 1)

At 60 DAS, Treatment T_2 showed the highest efficacy against Alternaria alternata, with the lowest pooled disease incidence (15.85%) and the highest percent efficacy of disease control (PEDC) at 59.51%. Treatment T_3 also performed well with a pooled PEDC of 49.52%. Conversely, the control (T7) had the highest disease incidence (39.19%) and no efficacy. Treatments T_4, T_5, and T_6 had lower efficacy, with pooled PEDCs ranging from 14.36% to 34.28%. Statistical analysis confirmed significant differences, highlighting T2 as the most effective treatment for disease control. (Table 2)

At 90 DAS, Treatment T_2 demonstrated the highest efficacy, exhibiting the lowest pooled disease incidence rate at 24.17% and achieving the greatest percentage of disease control. (PEDC) at 53.69%. Treatment T_3 also showed notable efficacy, with a pooled PEDC of 43.04%. In contrast, the control (T_7) had the highest disease incidence (52.20%) and no efficacy. Treatments T_4, T_5, and T_6 exhibited lower efficacy, with PEDCs ranging from 13.77% to 34.85%. Statistical analysis indicated significant differences, confirming T_2 as the best treatment for controlling Alternaria alternata. (Table 3)

The seed yield results for 'SKAU-M-86' during Kharif 2022 and 2023 show significant variations among treatments. Treatment T_2 yielded the highest average seed yield of 1065.65 kg/ha, surpassing all other treatments. Treatment T_3 also performed well with a pooled yield of 945.45 kg/ha. Intermediate yields were observed in Treatments T_1, T_5, and T_6, with yields ranging from 755.52 to 898.97 kg/ha. Treatment T_4 Yielded the minimal outcome yield of 653.12 kg/ha, while the control (T_7) had the lowest yield overall at 553.05 kg/ha. Statistical analysis highlights Treatment T_2 as the most effective in increasing seed yield. (Table 4)

Conclusion

An investigation into the impact of various integrated disease management strategies on the seed yield of 'SKAU-M-86' during *Kharif* 2022 and 2023 demonstrated significant differences among treatments. Treatment T_2 consistently produced the highest seed yield, with an average of 1065.65 kg/ha, indicating its superior efficacy in enhancing yield. Treatment T_3 also showed good performance with a yield of 945.45 kg/ha. Treatments T_1, T_5, and T_6 resulted in moderate yields, whereas Treatment T_4 and the control (T_7) had the lowest yields. These findings underscore the importance of selecting effective disease management strategies, with Treatment T_2 being particularly recommended for maximizing seed yield in 'SKAU-M-86'.

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