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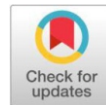
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Evaluation of chemical and biological control strategies against leaf spot in niger incited by *Alternaria alternata*

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ABSTRACT

Niger (*Guizotia abyssinica* L.) is a traditional minor oilseed crop predominantly cultivated in tribal regions of India. Under conducive environmental conditions, niger is susceptible to various diseases, among which leaf spot caused by *Alternaria alternata* is one of the most predominant, leading to significant yield losses. In recent years, the area under niger cultivation has increased in the Konkan region of Maharashtra; however, there is a limited availability of crop-specific fungicidal recommendations. Additionally, there is a pressing need for effective, eco-friendly disease management alternatives suitable for smallholder farming systems. Therefore, the present investigation was undertaken to evaluate the efficacy of fungicides and bio-agents against leaf spot disease, both under in vitro and field conditions. In the in vitro studies, complete inhibition of *Alternaria alternata* was observed with treatments of Hexaconazole 5% EC at 0.1%, Carbendazim 12% + Mancozeb 63% WP at 0.2%, and Hexaconazole 4% + Zineb 68% WP at 0.1%. Among the seven bio-agents tested, *Pseudomonas fluorescens* strain-4 proved to be the most effective, recording the least mycelial growth (22.16 mm) and the highest inhibition (75.36%) of *A. alternata*. The most promising treatments from the in vitro evaluation were subsequently selected for field trials, integrated with seed treatment. All tested treatments were significantly superior to the untreated control. The lowest disease severity (6.87%) and the highest disease reduction (84.94%) were achieved with seed treatment using Captan 50% WP at 4 g/kg, combined with two foliar sprays of Tebuconazole 50% WG + Trifloxystrobin 25% WG at 0.05%. A comparable level of control was obtained with seed treatment using Captan 50% WP at 4 g/kg, followed by two sprays of Carbendazim 12% + Mancozeb 63% WP at 0.15%, which resulted in 8.70% disease severity and 80.93% disease reduction compared to the control.

Keywords: *Niger* (*Guizotia abyssinica*), Leafspot, *Alternaria alternata*, Fungicides, Bio-agents, Field efficacy, Disease management

INTRODUCTION

Niger (*Guizotia abyssinica* L.), a traditional minor oilseed crop cultivated in India, belongs to the family Compositae with the chromosome number of $2n = 30$. In various regions of the nation, it is referred to by various names, including ramtil in Hindi, ramtil or kharsani in Gujarati, khurasani in Marathi, echelle in Kannada, payellu in Tamil, verrinuvvulu in Telugu, alashi in Oriya, sarguza in Bengali, ramtil in Punjab, and sorguja in Assamese [9]. Along with other crops like finger millet, it is thought to have been brought to India by Ethiopian immigrants sometime in the third millennium BC [2].

Despite being classified as a minor oilseed, niger is important since it contains 18–24% protein and 32–40% high-quality oil [8]. The niger oil contains essential amino acids among these are leucine, lysine, isoleucine, threonine, and tryptophan are used in cooking, painting, anointing, and lubricating equipment [6].

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Niger seeds can be dried and used with flour to form delectable cakes, or they can be eaten fried and used as a condiment. The oil tastes like desi ghee and free from any toxins. Additionally, niger seed oil is used to treat scabies and burns. The seeds contain nearly 40% oil, rich in fatty acids such as linoleic acid (45.4 to 65.8%), oleic acid (13.4 to 39.3%), palmitic acid (8.2 to 9.4%), and stearic acid (5.0 to 7.5%). After oil extraction, the oil cake consists of approximately 24% protein and 24% crude fibers. Worldwide, niger is grown to a limited extent in Ethiopia, South Africa, East Africa, Zimbabwe, India, and the West Indies. In India, niger is mostly grown in the tribal regions of Madhya Pradesh, Odisha, Bihar, Karnataka, Maharashtra, Gujarat and Andhra Pradesh.

In India, niger is mainly grown by marginal, small, and medium farmers for home consumption as well as serving as a source of income. But the production and productivity of niger are affected by many factors which are responsible for low crop yield, of which biotic factor is the major one. Among the biotic agents, leaf spot/blight caused by *Alternaria alternata* is a major devastating disease to the niger in India and also reduces the yield and oil quality [5]. This diseases cause heavy damage to up to 35-40 percent to this crop and reduce its seed yields by up to 20-30 percent.

Considering the increasing importance of niger, the present studies conducted on "Evaluation of chemical and biological control strategies against *Alternaria alternata* causing leaf spot in niger".

MATERIAL AND METHODS

Location

The present investigation was carried out in the laboratory and in the field during *Kharif* 2024. Field experiments were conducted at the Department of Plant Pathology, College of Agriculture, Dapoli while laboratory experiments were carried out at the Department of Plant Pathology, College of Agriculture, Dapoli, Dr. B. S. K. K. V., Dapoli.

Fungicides

Various fungicides used for the study belonging to the different fungicidal groups were purchased from an authorized store of Krishi Seva Kendra from Dapoli City.

Bio-agents

Pure strains of fungal bio-agents viz., *Trichoderma harzianum*, *T. longibrachiatum*, *T. viride*, and *T. koningii* and bacterial bio-agent, *Pseudomonas fluorescens* strain-1, *Pseudomonas fluorescens* strain-4 and *Pseudomonas fluorescens* strain-5 used in present study were obtained from Dr. B. S. K. K. V- AIMCCC, Department of Plant Pathology, College of Agriculture, Dapoli.

Isolation of *Alternaria alternata*

The diseased plant showing typical leaf spot symptoms were collected from niger growing fields from the University farm and causal fungus was isolated from the infected tissues and cultured aseptically on potato dextrose medium by standard tissue isolation technique. The isolated fungus was identified based on morphological and cultural characteristics of the causal fungus on the host as well as in the culture grown on PDA.

In vitro efficacy of different fungicides against *A. alternata*.

Different fungicides (Carbendazim 50% WP @ 0.1%, Azoxystrobin 23% SC @ 0.05%, Mancozeb 75% WP @ 0.2%, Copper oxychloride 50% WP @ 0.25%, Carbendazim 12% + Mancozeb 63% WP 75 WP @ 0.15%, Hexaconazole 4% + Zineb 68% W 72% WP @ 0.1% and Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05%) were evaluated under *in vitro* condition against *A. alternata* by Poisoned Food Technique (PFT). The diameter of the mycelial growth (mm) of the pathogen was measured after 7 days of incubation. Each treatment was replicated thrice in a Completely Randomized Design. The antifungal activity were recorded in terms of inhibition of mycelial growth (%) calculated using following formula [12].

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of the test fungus in untreated control plate

T = Growth (mm) of the test fungus in treated plates

In vitro evaluation of bio-agents against *A. alternata*

A total of seven fungal and bacterial bio-agents viz., *Trichoderma harzianum*, *T. longibrachiatum*, *T. viride* and *T. koningii* and bacterial bio-agent, *Pseudomonas fluorescens* Strain-1, *Pseudomonas fluorescens* Strain-4 and *Pseudomonas fluorescens* Strain-5 were evaluated against *A. alternata* by using Dual culture technique [1] and using PDA as a basal medium.

Each treatment was replicated thrice in Completely Randomized Design. The per cent inhibition of mycelial growth (%) calculated using following formula.

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of the test fungus in untreated control plate

T = Growth (mm) of the test fungus in treated plates

Management of leaf spot of niger incited by *A. alternata* using fungicides and bio-agents under field condition.

The fungicides and bioagents found effective in *in vitro* studies were evaluated in field conditions along with seed treatment with fungicides and bioagents. The field experiment was laid out in Randomized Block Design (RBD) with three replications at the experimental farm of the Department of Plant Pathology, College of Agriculture, Dapoli, Dist. Ratnagiri during *Kharif* 2024 on the local variety of niger under natural epiphytotic conditions. Seed treatment was given before the sowing. The seeds of the niger variety Cv. Local susceptible to *Alternaria* leaf spot disease were sown on 16th August, .2024 in the field. The recommended package of practices and protective irrigation was given as and when required. Two foliar sprays of respective fungicides and bioagents were applied: the first at the onset of disease symptoms (4th Oct.2024), and the second spray 15 days later (16th Oct. 2024). The treatment details are as follows.

Treatments Details:

| Tr. No. | Treatments details |
|----------------|---|
| T ₁ | Seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Azoxystrobin 23 % SC @ 0.05 % |
| T ₂ | Seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Carbendazim 12% + Mancozeb 63% WP @ 0.15 % |
| T ₃ | Seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Hexaconazole 4 % + Zineb 68% (72% WP) @ 0.1% |
| T ₄ | Seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Tebuconazole 50 % WG + Trifloxystrobin 25 % WG @ 0.05 % |
| T ₅ | Seed treatment with <i>Trichoderma harzianum</i> @ 10 g/kg + two sprays of <i>Trichoderma harzianum</i> @ 10g/L. |
| T ₆ | Seed treatment with <i>Pseudomonas fluorescens</i> strain-4 @ 5 g/kg + two sprays of <i>Pseudomonas fluorescens</i> strain-4 @ 5 g/L. |
| T ₇ | Control |

Observations on intensity of disease was recorded before spraying and at 15 days after each spraying by randomly selecting fifteen plants from each replications and graded as per 0 to 9 rating scale [4].

| Score | : | Description |
|-------|---|---------------------------------------|
| 0 | : | Healthy (Without spots) |
| 1 | : | < 1% leaf area covered with spots |
| 3 | : | 1-10% leaf area covered with spots |
| 5 | : | 11-25% leaf area covered with spots |
| 7 | : | 26-50% leaf area covered with spots |
| 9 | : | > 50% of leaf area covered with spots |

The percent disease intensity (PDI) and percent disease control were calculated by using the following formula [13].

Percent disease intensity (PDI)

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{No. of leaves assessed} \times \text{Maximum disease grade value}} \times 100$$

Percent disease control (PDC)

$$\text{PDC} = \frac{\text{PDI in control plot} - \text{PDI in treatment plot}}{\text{PDI in control plot}} \times 100$$

Statistical analysis

The analysis of variance was done in completely randomized design and randomized block design and results were

presented at 1% and 5% levels of significance, respectively. The critical difference (CD) values were calculated to compare various treatment means.

RESULTS AND DISCUSSION

In vitro evaluation of different fungicides against *A. alternata*.

All the tested concentrations of different fungicides were found significantly superior over the control against *A. alternata* (Table 1 and Plate I). Complete inhibition of *Alternaria alternata* was observed in treatments T₃ (Hexaconazole 5% EC @ 0.1%), T₆ (Carbendazim 12% + Mancozeb 63% WP @ 0.15%), and T₇ (Hexaconazole 4% + Zineb 68% WP @ 0.1%). These were followed by T₈ (Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.05%) and T₂ (Azoxystrobin 23% SC @ 0.1%), which recorded mycelial growth of 11.16 mm and 12.86 mm, corresponding to 87.03% and 85.73% inhibition, respectively. Treatments T₅ (Copper oxychloride 50% WP @ 0.25%), T₄ (Mancozeb 75% WP @ 0.2%), and T₁ (Carbendazim 50% WP @ 0.1%) showed mycelial growth of 20.16 mm, 24.66 mm, and 30.83 mm, with inhibition of 77.59%, 72.58%, and 65.74%, respectively.

Our observations were in accordance with the findings of earlier studies of Prashant *et al.* [7]. They reported that Carbendazim 12% + Mancozeb 63% (75 WP) (0.2 %) was found highly effective in inhibiting the mycelial growth (14.39 mm) of *A. alternata* followed by Carbendazim 50 WP (0.1%) (17.01 mm). Similarly, Carboxin 37.5% + Thiram 37.5% DS @ 0.3% inhibit 100% mycelial growth of *Alternaria alternata* followed by Carbendazim 12% + Mancozeb 63% 75 WP (93.15%), Copper oxychloride 50% WP (92.22%), Propineb 70% WP (90.00%) and Pyraclostrobin 20% WG (80.37%) [10].

In vitro evaluation of bio-agents against *A. alternata*.

All the evaluated bio-agents were significantly effective in inhibiting the mycelial growth of *Alternaria alternata* (Table 2 and Plate II). Among the seven bio-agents tested, *Pseudomonas fluorescens* strain - 4 was found most effective which showed least mycelial growth (22.16 mm) and the highest inhibition (75.36%) of *A. alternata*. This was followed by *Trichoderma harzianum*, *T. viride*, *T. longibrachiatum*, and *T. koningii*, which recorded mycelial growth of 25.50, 27.83, 28.83, and 29.83 mm, corresponding to 71.66%, 69.07%, 67.96%, and 66.85% inhibition, respectively.

Similar results were reported by earlier scientist [10], they studied *in vitro* efficacy of various bioagents by dual culture method against *Alternaria alternata* causing leaf spot disease of niger. Among the tested bio-agents *Trichoderma reesei* was found superior recorded 75.00% mycelial growth inhibition of *Alternaria alternata* followed by *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Trichoderma asperellum* with 65.36, 60.36 and 58.88 percent mycelial growth inhibition, respectively. Ghule *et al.* [3] evaluated the *in vitro* efficacy of four bio-agents inciting leaf blight of chrysanthemum. The results revealed that maximum mycelial growth inhibition of *Alternaria alternata* was observed in *Trichoderma harzianum* (79.25%) which was followed by *Trichoderma asperellum* (77.15%), *Pseudomonas fluorescens* (51.58%) and *Bacillus subtilis* (45.87%).

Management of leaf spot of niger incited by *A. alternata* using fungicides and bio-agents under field conditions.

All the tested fungicides and bioagents under field conditions were found effective in control of leaf spot disease of niger incited by *A. alternata* (Table 3).

Minimum leaf spot severity (6.87%) and maximum per cent disease reduction (84.94%) were recorded in the treatment T₄ (seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Tebuconazole 50% WG + Trifloxystrobin 25% WG @ 0.05 %). Treatment T₂ (seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Carbendazim 12% + Mancozeb 63% WP @ 0.15 %) was found at par with treatment T₄ which recorded 8.70 percent disease severity and 80.93 percent disease reduction over control. This was followed by treatment T₃ (seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Hexaconazole 4% + Zineb 68% (72% WP) @ 0.1%), T₁ (seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Azoxystrobin 23% SC @ 0.05 %) and T₆ (seed treatment with *Pseudomonas fluorescens* @ 5 g/kg + two sprays of *Pseudomonas fluorescens* strain - 4 @ 5 g/L) with 11.75 %, 14.33 % and 17.10% disease severity with 74.25%, 68.60% and 62.53% disease reduction over control, respectively. Treatment T₅ (seed treatment with *Trichoderma harzianum* @ 10 g/kg + two sprays of *Trichoderma harzianum* @ 10g/L.) was found least effective compared to the other treatments with 21.17 % disease severity and 53.61% disease reduction over control.

The present findings were in accordance with the earlier studies of Sandipan *et al.* [9], they evaluated the fungicides for the management of *Alternaria* leaf spot disease niger under field conditions. The results revealed that the least incidence of *Alternaria* leaf spot (19.08 %) was observed in treatment T₆ (Two foliar sprays of Carbendazim 12% + Mancozeb 63% @ 0.2%), followed by the treatment T₁ (Two foliar sprays of Carbendazim 50 WP @ 0.1%) with 19.00 percent disease severity. Venkataramanamma *et al.* [11] also evaluated the fungicides for the management of *Alternaria* leaf spot/ blight of sunflower caused by (*Alternaria helianthi* Hansf) under field conditions. The results revealed that among different treatments, the treatment T₃ (Seed treatment with Carbendazim 12% + Mancozeb 63% @ 2g/kg seed followed by two foliar sprays with Trifloxystrobin 25%+ Tebuconazole 50% @ 0.25 g/l) has recorded low severity (37.00%) with a higher yield of 1382 kg/ha, which was followed by the treatment T₁ (Seed treatment with Carbendazim 12% + Mancozeb 63% WP @ 2 g/kg seed followed by two foliar sprays with Difenconazole 25% + Propiconazole 25% @ 0.3 ml/l) which has recorded 40.95% disease severity with yield of 1363 kg/ha compared to the control (53.38% and 1200 kg/ha, respectively).

CONCLUSION

- The fungicides Hexaconazole 5% EC, Carbendazim 12% + Mancozeb 63% WP, Hexaconazole 4% + Zineb 68% WP, and Tebuconazole 50% WG + Trifloxystrobin 25% WG @ 0.05%, along with the bio-agents *Pseudomonas fluorescens* strain-4 and *Trichoderma harzianum*, were found to be effective against *Alternaria alternata* under *in vitro* conditions.
- Seed treatment with Captan 50% WP @ 4 g/kg, followed by two foliar sprays of Tebuconazole 50% WG + Trifloxystrobin 25% WG @ 0.05% at 15 day intervals after disease initiation, and seed treatment with Captan 50% WP @ 4 g/kg followed by two sprays of Carbendazim 12% + Mancozeb 63% WP @ 0.15%, were found to be the most effective treatments for managing leaf spot disease of niger caused by *A. alternata* under field conditions.

FUTURE SCOPE OF THE STUDY

The study provides a strong foundation for formulating region-specific IDM modules for leaf spot of niger, combining seed

treatment, bio-agents, and need-based fungicide applications to reduce disease pressure and input costs. Further research can focus on refining and validating bio-agent-based management practices and evaluating fungicide residues on niger seeds to ensure food safety and environmental sustainability.

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CONFLICT OF INTEREST: All authors declare that they have no conflicts of interest.

Table 1: In vitro efficacy of different fungicides against *Alternaria alternata*

| Tr. No. | Fungicide | Conc. (%) | Mean colony diameter (mm)* | Per cent inhibition |
|----------------|---|-----------|----------------------------|---------------------|
| T ₁ | Carbendazim 50% WP | 0.1% | 30.83 | 65.74 |
| T ₂ | Azoxystrobin 23% SC | 0.05% | 12.83 | 85.73 |
| T ₃ | Hexaconazole 5% EC | 0.1% | 0.00 | 100.0 |
| T ₄ | Mancozeb 75% WP | 0.2% | 24.66 | 72.58 |
| T ₅ | Copper oxychloride 50% WP | 0.25% | 20.16 | 77.59 |
| T ₆ | Carbendazim 12% + Mancozeb 63% (75 WP) | 0.15% | 0.00 | 100.0 |
| T ₇ | Hexaconazole 4% + Zineb 68% (72% WP) | 0.1% | 0.00 | 100.0 |
| T ₈ | Tebuconazole 50% + Trifloxystrobin 25% WG | 0.05% | 11.66 | 87.03 |
| T ₉ | Control | - | 90.00 | - |
| | S.E.m ± | | 0.52 | |
| | C.D. at 1% | | 2.01 | |

Table 2: In vitro efficacy of different bio-agents against *Alternaria alternata*

| Tr. No. | Bio agents evaluated | Mean colony diameter (mm)* | Per cent inhibition |
|----------------|--|----------------------------|---------------------|
| T ₁ | <i>Trichoderma harzianum</i> | 25.50 | 71.66 |
| T ₂ | <i>Trichoderma koningii</i> | 29.83 | 66.85 |
| T ₃ | <i>Trichoderma longibrachiatum</i> | 28.83 | 67.96 |
| T ₄ | <i>Trichoderma viride</i> | 27.83 | 69.07 |
| T ₅ | <i>Pseudomonas fluorescens</i> strain -1 | 32.16 | 64.25 |
| T ₆ | <i>Pseudomonas fluorescens</i> strain -4 | 22.16 | 75.36 |
| T ₇ | <i>Pseudomonas fluorescens</i> strain -5 | 29.83 | 66.84 |
| T ₈ | Control | 90.00 | - |
| | S.E.m ± | 0.65 | |
| | C.D. at 1% | 2.69 | |

Table 3: Field efficacy of fungicides and bio-agents against leaf spot disease of niger incited by *Alternaria alternata*

| Tr. No. | Treatment | Per cent Disease Intensity (PDI)* | | | Per cent Disease Control |
|----------------|--|-----------------------------------|--------------------------------|--------------------------------|--------------------------|
| | | Before spraying | After 1 st spraying | After 2 nd spraying | |
| T ₁ | Seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Azoxystrobin 23% SC @ 0.05 % | 2.05 (6.17) * | 5.81 (10.45) | 14.33 (16.68) | 68.60 |
| T ₂ | Seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Carbendazim 12% + Mancozeb 63% WP @ 0.15 % | 1.75 (5.70) | 2.30 (6.51) | 8.70 (12.86) | 80.93 |
| T ₃ | Seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Hexaconazole 4% + Zineb 68% (72% WP) @ 0.1% | 1.88 (5.91) | 4.77 (9.46) | 11.75 (15.03) | 74.25 |
| T ₄ | Seed treatment with Captan 50 % WP @ 4 g/kg + two sprays of Tebuconazole 50% WG + Trifloxystrobin 25% WG @ 0.05 % | 1.10 (4.52) | 3.05 (7.50) | 6.87 (11.38) | 84.94 |
| T ₅ | Seed treatment with <i>Trichoderma harzianum</i> @ 10 g/kg + two sprays of <i>T. harzianum</i> @ 10g/L. | 4.35 (9.02) | 9.81 (13.69) | 21.17 (20.54) | 53.61 |
| T ₆ | Seed treatment with <i>Pseudomonas fluorescens</i> strain -4 @ 5 g/kg + two sprays of <i>P. fluorescens</i> strain-4 @ 5 g/L | 3.17 (10.24) | 7.39 (15.77) | 17.10 (24.42) | 62.53 |
| T ₇ | Control | 6.51 (14.78) | 21.38 (27.53) | 45.64 (42.50) | |
| | S.E.m ± | 0.22 | 0.47 | 0.63 | |
| | C. D. at 5% | 0.69 | 1.47 | 1.94 | |

Figures in parenthesis are arc-sin transformed values**Plate I: In vitro efficacy of fungicides against *A. alternata*Plate II: In vitro efficacy of bio-agents against *A. alternataREFERENCES**

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