***Short Research Article***

**In vitro and in-vivo evaluation of different fungicides against *Alternaria brassicae* causing Alternaria blight disease of Mustard**

**Abstract**

Mustard is the second most important oilseed crop in India after groundnut and contributes about 35% in the total vegetable oil production. Mustard crop is sensitive to numerous abiotic and biotic stresses, which affects the production and productivity drastically. Alternaria blight disease of mustard is one the most destructive disease which affects all growth stages and cause huge economical loss. Considering the severity and significance of damage caused by this disease, an *in vitro* and *in vivo* evaluation of eight fungicide was done against *A. brassicae*. It was found that Propiconazole 25 EC showed complete mycelial growth inhibition at all the three concentrations i.e., 100, 200 and 300 ppm whereas, Tebuconazole 25.9 EC recorded complete mycelial growth inhibition at 200 and 300 ppm. Among the tested fungicides, the minimum percent disease severity on leaves was observed in crops sprayed with Azoxystrobin 12.5% + Tebuconazole 12.5% SC followed by Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 235 SC.

**Keywords**: Alternaria blight disease, Fungicide, Poisoned food technique, Percent inhibition, Disease severity,

**1.Introduction**

Mustard is an important oilseed crop and ranks third after soybean and palm oil in the world. In India, it is grown in an area of 6.23 mha with production and productivity of 9.34 mt and 1499 kg/ha respectively. Out of all the Rapeseed-mustard crops cultivated in India, mustard occupies about 75-80% of the total area occupied by Rapeseed-mustard (Annual report ICAR-DRMR, 2018-19). It is an important source of oil in India and contributes about 35% in the total vegetable oil production. It is the second most essential oilseed crop after groundnut and was significant contributor of the “Yellow Revolution” launched in 1986-1987 which aimed to increase edible oil production. Mustard is sensitive to numerous biotic and abiotic stresses which further widens the existing gap between potential yield and yield realized at the farmer’s field.

Among the major diseases affecting mustard, alternaria blight is one of the most destructive disease which occurs frequently throughout the world and infects all growth stages causing huge economical loss. An average yield loss of 35-38% has been reported from India (Kolte, 1985a,b; Kolte et al. 1987; Chattopadhyay, 2009)**.** The alternaria blight infected seeds of rapeseed-mustard are reported to suffer a loss of 14.6-36% in oil content over the seeds of healthy plants (Ansari et al. 1988). In addition to direct yield loss, it also affects the quality and germination of seed by reducing the size of seed, colour and oil content (Kaushik et al.1984).

Alternaria species are either saprophyte on organic substrate or parasites on living plants (Singh et al. 2017). Symptoms of alternaria blight of mustard is characterized by formation of spots on leaves, stem and pods. Symptom in seedlings appears as small light brown lesion on cotyledon and hypocotyls and dark stem lesions appear immediately after germination of seedling which often results in damping-off or stunted seedlings**.** On adult plants, symptoms are first visible on lower leaves, which appear as black points that later enlarges into prominent, round concentric rings that are grey in colour. On siliqua and stem, brownish-black spots appear with grey centre. The primary infection starts from either diseased stubbles or neighbouring volunteer crops whereas, secondary infection takes place through conidia formed on diseased leaves or pods (Kumar et al. 2016).The alternaria blight infection on leaves and siliqua reduces the photosynthetic area drastically (Meena et al. 2010). Infection on siliqua affects the normal seed development, its weight, colour and percent oil content and seed quality.

**2. Materials and methods**

**2.1. Isolation of the pathogen**

Mustard leaf samples infected with Alternaria blight were collected from Bihar Agricultural University farm, Sabour, Bhagalpur.Infected leaf samples showing typical characteristic symptom of Alternaria blight were used for isolation of *A. brassicae*. Leaf samples were first washed under running tap water and then blot dried. Small bits of leaf with a healthy plus diseased portion were taken and sterilized with 4% sodium hypochloride for 1 min followed by three times washing in distilled water. The pieces were blot dried using sterilized blotting paper and transferred on solidified RRSA (Radish root sucrose agar) media and incubated for 7 days at 20 ± 2 °C. Sub-culturing was done using culture portion showing typical *A. brassicae* colony characteristics. Pure culture was obtained through single spore isolation.

**2.2. Pathogenicity test**

Pathogenecity test was conducted in laboratory using susceptible *Brassica juncea* variety Varuna. A spore suspension of 104 spores/ml was used for inoculation on 25 day old plant (Kumar et al. 2014). The fungus was re-isolated from the infected leaves showing typical alternaria blight characteristics and maintained in RRSA media. The re-isolated fungus was similar to the original isolate of the pathogen in morphology. Thus, Koch’s postulate was satisfied.

**2.3. In vitro evaluation of different fungicides against *Alternaria brassicae***

Evaluation of eight fungicide belonging to different groups and showing different mode of action was taken and tested viz., Mancozeb 75% WP, Propiconazole 25 EC, Tebuconazole 25.9% EC, Azoxystrobin 23% SC, Metalaxyl 8% + Mancozeb 64% WP, Tebuconazole 10% + Sulphur 65% WG, Azoxystrobin 12.5% + Tebuconazole 12.5%, Tebuconazole 50% + Trifloxystrobin 25% WG at three different concentration of 100 ppm, 200 ppm and 300 ppm. Different treatments were tested against the pathogen under in vitro condition to find out the relative efficacy in inhibiting the growth of culture by the “Poisoned Food Technique”. A stock solution of 10000 ppm of each fungicide (treatment) was prepared by dissolving the required amount of it in a measured volume of sterilized distilled water. Then, the calculated amount of stock solution was added into warm RRSA media so as to obtain the required fungicide concentrations. It was then poured into sterilized petriplates and allowed to solidify. With the help of a sterilized cork borer, 5 mm bits of 10 days old *Alternaria* culture were taken. It was then inoculated at the center of poisoned media in three replications of each concentration. The fungal bits were inversely placed so that it comes in direct contact with the media. The inoculated Petri plates were kept in BOD incubator at 20 ± 2°C. Control was maintained without mixing the media with any fungicide and just inoculating it with test pathogen.

The efficacy of different chemicals was observed by measuring the colony diameter in millimeters (mm). Percent inhibition over control was assessed using the following formula (Vincent, 1947)

**R= [( C-T ) / C ] \* 100**

**Where,** R = Percent inhibition (%)

C = Radial growth of pathogen colony in control (mm)

T = Radial growth of pathogen colony in treatment (mm)

**2.4. In vivo evaluation of fungicides against *Alternaria brassicae***

The experiment was conducted with mustard variety- Rajendra sufalam to evaluate eight different fungicide for the management of Alternaria blight disease (Table 1). The fungicides were sprayed twice, first spray was given at the onset of disease incidence and the second spray was given 15 days after 1st spray under field condition with the help of Knapsack sprayer.

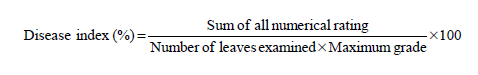
**Table 1: List of fungicides and their concentrations evaluated under field condition for the management of alternaria blight disease**

|  |  |  |
| --- | --- | --- |
| **S. No** | **Fungicides** | **Dosage** |
| 1 | Mancozeb 75% WP | 2gm/lit |
| 2 | Propiconazole 25 EC | 1ml/lit |
| 3 | Tebuconazole 25.9% EC | 1ml/lit |
| 4 | Azoxystrobin 23% SC | 2.5ml/lit |
| 5 | Metalaxyl 8% + Mancozeb 64% WP | 2gm/lit |
| 6 | Tebuconazole 10% + Sulphur 65% WG | 1gm/lit |
| 7 | Azoxystrobin 12.5% + Tebuconazole 12.5% SC | 3gm/lit |
| 8 | Tebuconazole 50% + Trifloxystrobin 25% WG | 1gm/lit |
| 9 | Control |  |

**2.5. Disease severity on leaves**

Disease severity (%) was calculated on the basis of 0-5 rating scale (Conn et al.1990).The details of rating scale are given in the table 2. For observation, sixth leaf from the top of ten randomly selected and tagged plants were taken plant. Disease severity was recorded at 40, 55 and 70 DAS at 15 days interval.

On the basis of 0-5 rating scale (table 2), disease severity (%) was calculated by using the following formula by Mckinney, 1923.

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|  |  |
| --- | --- |
| **Grade** | **Extent of infection** |
| 0 | No symptom |
| 1 | 1-10% leaf area infection |
| 2 | 11-25% leaf area infection |
| 3 | 26-50% leaf area infection |
| 4 | 51-75% leaf area infection |
| 5 | >75% leaf area infection |

**Table 2: Detail of evaluation system for accessing Alternaria blight disease**

**3. Result**

The effect of fungicide on mycelial growth of *A.brassicae* was evaluated at 7 and 14 DAI at 100, 200 and 300 ppm. The percent mycelial growth inhibition was significant for all the concentrations. Tebuconazole 25.9 EC at 7 DAI showed 100 % inhibition of mycelial growth over control at all concentration while at 14 DAI 100% mycelial growth inhibition was observed only at 200 and 300 ppm. Propiconazole 25 EC showed 100 percent inhibition in both the observations (7 and 14 DAI) at all the concentrations i.e., 100, 200 and 300 ppm. The minimum percent inhibition was observed in Azoxystrobin 23% SC (24.04) followed by Metalaxyl 8% + Mancozeb 64% WP (26.33%) and Mancozeb 75% WP (28.62). At 14 DAI, the maximum percent mycelial growth inhibition over control at 200ppm was observed in Azoxystrobin 12.5% + Tebuconazole 12.5% SC (90.80%) followed by Tebuconazole 50% + Trifloxystrobin 25% WG (90.45%) and Tebuconazole 10% + Sulphur 65% WG (78.25%) whereas, the minimum percent inhibition was observed in Azoxystrobin 23% SC (30.15%) followed by Mancozeb 75% WP (32.82%) and Metalaxyl 8% + Mancozeb 64% WP (35.86%). It was observed that Azoxystrobin 12.5% + Tebuconazole 12.5% SC and Tebuconazole 50% + Trifloxystrobin 25% WG were statistically at par (Table 3).

**Table 3: Effect of fungicide on percent mycelial growth inhibition of *Alternaria brassicae***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Percent growth inhibition** | | | | | |
| **100 ppm** | | **200 ppm** | | **300 ppm** | |
| **7 DAI** | **14 DAI** | **7 DAI** | **14 DAI** | **7 DAI** | **14 DAI** |
| Mancozeb 75% WP | 51.43  (45.80)\* | 28.62  (32.33) | 54.71  (47.68) | 32.82  (34.94) | 57.58  (49.34) | 36.63  (37.23) |
| Propiconazole 25 EC | 100.00  (90.00) | 100.00  (90.00) | 100.00  (90.00) | 100.00  (90.00) | 100.00  (90.00) | 100.00  (90.00) |
| Tebuconazole 25.9EC | 100.00  (90.00) | 91.22  (72.74) | 100.00  (90.00) | 100.00  (90.00) | 100.00  (90.00) | 100.00  (90.00) |
| Azoxystrobin 23% SC | 45.49  (42.39) | 24.04  (29.34) | 47.94  (43.80) | 30.15  (33.29) | 51.84  (46.04) | 38.16  (38.14) |
| Metalaxyl 8% + Mancozeb 64%WP | 52.05  (46.15) | 26.33  (30.86) | 55.12  (47.92) | 35.86  (36.77) | 58.60  (49.93) | 39.69  (39.03) |
| Tebuconazole 10% + Sulphur 65% WG | 74.59  (59.71) | 47.70  (43.67) | 84.63  (66.93) | 78.25  (62.19) | 90.16  (71.71) | 88.92  (70.57) |
| Azoxystrobin 12.5% + Tebuconazole 12.5% SC | 90.78  (72.31) | 90.07  (71.61) | 91.39  (72.92) | 90.80  (72.31) | 100.00  (90.00) | 91.60  (73.13) |
| Tebuconazole 50% + Trifloxystrobin 25% WG | 89.75  (71.31) | 88.17  (69.86) | 90.78  (72.30) | 90.45  (71.98) | 92.00  (73.55) | 91.21  (72.74) |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C.D.at 1% | 1.12  (0.92) | 1.18  (0.87) | 1.54  (1.19) | 2.06  (1.34) | 1.57  (1.08) | 1.97  (1.46) |
| S.Em.± | 0.37  (0.31) | 0.39  (0.29) | 0.51  (0.40) | 0.69  (0.45) | 0.53  (0.36) | 0.66  (0.49) |
| C.V.% | 0.96  (0.92) | 1.24  (1.03) | 1.28  (1.16) | 1.92  (1.42) | 1.26  (1.00) | 1.75  (1.49) |

\*Data in the parenthesis are angular transformed values

**3.1. Effect of fungicide on alternaria blight disease severity at different stages of growth**

The percent disease severity on leaf increased progressively with age of plant and was observed in all the treatments. Minimum disease severity was observed in Azoxystrobin 12.5% + Tebuconazole 12.5% SC (5.67), Azoxystrobin 23% SC and Tebuconazole 50 % + Trifloxystrobin 25% WG (6.00). All the treatments showed significant difference at 55 and 70 DAS. At 55 and 70 DAS, the minimum disease severity was observed in Azoxystrobin 12.5% + Tebuconazole 12.5% SC (8.67 and 9.33) respectively. It was followed by Tebuconazole 50 % + Trifloxystrobin 25% WG (9.0 and 11.33) and Azoxystrobin 23% SC (10.67 and 13.33) at 55 and 70 DAS respectively. Azoxystrobin 12.5% + Tebuconazole 12.5% SC and Tebuconazole 50 % + Trifloxystrobin 25% WG and Azoxystrobin 23% SC were found statistically at par at 55 DAS while at 70 DAS, Azoxystrobin 12.5% + Tebuconazole 12.5% SC was statistically different with Azoxystrobin 23% SC. At 55 DAS and 70 DAS, the maximum percent severity was observed in control (28.67 and 45.33) respectively. Maximum reduction over control and minimum average disease severity was observed in Azoxystrobin 12.5% + Tebuconazole 12.5% SC (71.13 and 7.89) (Table 4).

**Table 4: Effect of fungicide on alternaria blight disease severit at different stages of growth**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Dosage** | **Percent disease severity** | | | **Mean** | **Reduction over control (%)** |
| **40**  **DAS** | **55 DAS** | **70 DAS** |
| **Mancozeb 75% WP** | 2g/lit | 8.00  (16.42)\* | 16.00  (23.54) | 30.67  (33.61) | 18.22  (24.54) | 33.33 |
| **Propiconazole 25 EC** | 1ml/lit | 8.67  (17.09) | 13.33  (21.32) | 24.67  (29.76) | 15.56  (22.76) | 43.07 |
| **Tebuconazole 25.9% EC** | 1ml/lit | 6.33  (14.56) | 11.33  (19.65) | 15.33  (23.03) | 11.00  (19.09) | 59.75 |
| **Azoxystrobin 23% SC** | 2.5ml/lit | 6.00  (14.14) | 10.67  (19.04) | 13.33  (21.40) | 10.00  (18.21) | 63.41 |
| **Metalaxyl 8% + Mancozeb 64% WP** | 2gm/lit | 7.33  (15.67) | 18.00  (25.07) | 28.67  (32.36) | 18.00  (24.39) | 34.14 |
| **Tebuconazole 10% + Sulphur 65% WG** | 1gm/lit | 7.67  (16.02) | 12.00  (20.26) | 14.00  (21.93) | 11.22  (19.43) | 58.95 |
| **Azoxystrobin 12.5% + Tebuconazole 12.5% SC** | 3gm/lit | 5.67  (13.72) | 8.67  (17.09) | 9.33  (17.76) | 7.89  (16.22) | 71.13 |
| **Tebuconazole 50% + Trifloxystrobin 25% WG** | 1gm/lit | 6.00  (14.14) | 9.00  (18.43) | 11.33  (19.65) | 8.78  (17.10) | 67.87 |
| **Control** | - | 8.00  (16.34) | 28.67  (32.36) | 45.33  (42.31) | 27.33  (30.36) | - |
| **S.Em.±** | - | 0.71  (0.79) | 0.95  (0.79) | 0.68  (0.51) | - | - |
| **C.D. at 5%** | - | NS | 2.85  (2.39) | 2.03  (1.55) | - | - |
| **C.V.(%)** | - | 17.29  (8.90) | 11.50  (6.27) | 5.47  (3.31) | - | - |

\*Data in the parenthesis are angular transformed values

**4. Discussion**

It can be concluded that Propiconazole is superior among all the treatments followed by Tebuconazole 25.9 EC, Azoxystrobin 12.5 % + Tebuconazole 12.5 % and Tebuconazole 50 % + Trifloxystrobin 25 % WG. The fungicide Propiconazole 25 EC and Tebuconazole 25.9 EC found superior in the evaluation belongs to Triazole group of fungicide and are excellent systemic fungicide against ascomycetes. Their effectiveness against *A. brassicae* could be attributed to their mode of action. These fungicides interfere with normal ergosterol biosynthesis mechanism in the fungus cell and causes demethylation of C-14 which leads to accumulation of C-14 methyl sterols. Ergosterol plays a vital role in the fungus cell wall formation, and is for crucial for its proper structure and functioning. Lack of normal sterol slows down and checks the growth of fungus.

The result corroborates the findings of Jackson and Kumar (2019) where Propiconazole showed 100 % inhibition of *A. brassicae* at much lower concentration i.e., 10, 25, 50, 75 and 100 ppm. Tebuconazole 250 EC + Trifloxystrobin WG 75, Propiconazole 25 EC, Tebuconazole 250 EC and Difenconazole 25 EC were highly effective in inhibiting the growth of *A.brassicae* (100 %) at 0.05 %, 0.1 % and 0.2 % concentration (Rajvanshi et al. 2020). Fungicides Propiconazole, Tebuconazole, Copper oxychloride and Hexaconazole are effective against *A*. *Brassicicola* at 0.05 %, 0.1 % and 0.2 % concentration (Kiran et al. 2018). In-vitro evaluation of six fungicide against *A. solani* showed that Propiconazole 25 EC showed maximum mycelial growth inhibition at 50 and 100 ppm whereas, it showed complete inhibition at 250 ppm followed by Carbendazim 50 WP (Husain et al. 2020). Six systemic fungicides were evaluated against *A. porri* at 0.5, 1, 10, 25, 100 and 200 µg/ml concentration, among the fungicides complete mycelial growth inhibition was recorded by Tebuconazole 25 EC at 25 µg/ml whereas, Tebuconazole 50 % + Trifloxystrobin 25 % WG and Propiconazole 25 EC showed complete mycelial growth inhibition at 100 and 200 µg/ml concentration respectively (Yadav et al. 2017).

Seed treatment with Apron @ 6g/kg and then fungicide application of Nativo @ 0.05 % was found most effective for the management of alternaria blight disease followed by Difenconazole @ 0.05 % and iprodione @ 0.2 % (Singh et al. 2018). The result corroborates with the findings of Pattanaik and Priyadarshini, (2020) who reported that plants treated with Azoxystrobin 11% + Tebuconazole 18.3 % was found most effective on early blight of tomato. The foliar spray of Azoxystrobin 11 % + Tebuconazole 18.3 % recorded least incidence of early blight disease (Palaiah et al. 2020).

**5. Summary and conclusion**

All the eight fungicides viz., Mancozeb 75% WP, Propiconazole 25 EC, Tebuconazole 25.9% EC, Azoxystrobin 23% SC, Metalaxyl 8% + Mancozeb 64% WP, Tebuconazole 10% + Sulphur 65% WG, Azoxystrobin 12.5% + Tebuconazole 12.5% SC, Tebuconazole 50% + Trifloxystrobin 25% WG significantly reduced mycelial growth of *A. brassicae* pathogen over control on RRSA media. Among all the eight fungicides evaluated *in vitro,* Propiconazole 25 EC showed complete mycelial growth inhibition at all the concentrations i.e., 100, 200 and 300 ppm at 7 and 14 Days after inoculation. Among the tested fungicides, the minimum mean percent disease severity on leaves was observed in crops sprayed with Azoxystrobin 12.5% + Tebuconazole 12.5% SC followed by Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 235 SC.

**DATA AVAILABILITY**All datasets generated or analyzed during this study are included in the manuscript.

**ETHICS STATEMENT**Not applicable.

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