In vitro evaluation of different fungicides against fenugreek powdery mildew caused by *Erysiphe polygoni*

Marakna Nisha Mansukbhbai, Golakiya Bhargav B and Kapadiya Haribhai J

**Abstract**

Powdery mildew caused by *Erysiphe polygoni* DC is one of the major constraints in the production of fenugreek. Farmers have to spray fungicides regularly for disease management. In order to find out the effective fungicides against *Erysiphe polygoni* experiment was carried out under *in vitro*. The relative efficacies of different fungicides were tested in different concentration. Various fungicides tested by spore germination inhibition technic for testing their efficacy, among them in non-systemic fungicide wettable sulphur, systemic fungicide propiconazole and ready-mix of fungicide tebuconazole + trifloxystrobin were found best against *E. polygoni* *in vitro* evaluation.

**Keywords:** Fenugreek, powdery mildew, fungicide, *Erysiphe polygoni*

**Introduction**

Powdery mildew has long been known as important disease of plants in all parts of world. Salmon (1900) \[14\] defined *Erysiphe polygoni* DC causing powdery mildew. Fenugreek (*Trigonella foenum graeceum* L.) is cultivated throughout India and belongs to the family *Leguminosae* (Balodi and Rao, 1991) \[2\]. India, annual production of fenugreek is 2.40 lakh tones from an area of 1.81 lakh ha has spread all over the country (Anon., 2019) \[1\]. Fenugreek is suffering from diseases like powdery mildew [*Erysiphe polygoni* DC.; *Leveillula Taurica* (Lev.)], downy mildew (*Peronospora trigonellae*), are serious diseases resulting 15 to 50 per cent seed yield losses (Kumawat and Shekhawat, 2015) \[11\]. It is a routine practice for farmers to spray fungicides onward from 45 days age to maturity of the crop to save seed yield from the epidemic of disease. There is complete failure of the crop, if disease occurs in epidemic form. Estimation of seed yield loss due to powdery mildew of fenugreek was 44.11 per cent under the field condition in saurashtra region (Chovatiya, 2010) \[4\]. The pathogen is an obligate parasite and multiply on only living host in the nature. Symptoms of powdery mildew initiated on 42-43 days after sowing. Initially white powdery growth mostly first on the upper then under surface of leaves. Gradually fungal growth covers whole leaf surface with dirty white powdery coating as well as found on pod. It adapts well in environments with low humidity and moderate temperatures (Huang, 2000) \[7\].

**2. Materials and Methods**

**2.1. Spore germination inhibition of test pathogen**

Various criteria are used to determine the effect of different physiological agents on germination, of which the most frequently used as the percentage of spores that produce germ tubes. Water is the prime factor in any germinating medium and for many spores is the only substance necessary to start germination. Its imbibition causes the first visible symptom of germination, the swelling of the spore, often to more than twice became its original size. Thus, absorption of water by spores resembles the imbibition activity of lyophilic colloids. This phenomenon, however, is dependent upon some vital mechanism of the spore, for dead spores do not swell and absorption varies with the viability of the spore. (David, 1950) \[3\]. Different chemicals viz., fungicides, insecticides, weedicides and phytoextracts were tested for their effect on spore germination inhibition of causal organism of powdery mildew by

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using spore germination inhibition technique. Powdery mildew disease infected leaves of fenugreek were collected from field. Spores were collected by gently rubbed sterilized brush on spotted part of leaves. So, spores were collected and added in sterilized distilled water. Muslin cloth bag was used for remove extra plant cells from spore suspension.

First prepared stock solutions of various fungicides with different level of concentration and then diluted the stock solution with distilled water for obtained required concentration in double strength. One drop of each chemical suspension and spore suspension was placed on a glass cavity slide, so concentration was obtained as per require for evaluation. The slides were then placed in petri plates lined with moistened coarse filter paper to provide sufficient humidity for germination of conidia for 24 hours at room temperature. After 24 hours, 48 hours and 72 hours intervals observation of spore germination inhibition was taken by using light microscope at 40x objective lens. The observations on the spore germination inhibition of the fungus in each treatment including check were taken and per cent inhibition was calculated on the basis of the difference between inhibition obtained in the treatment and control. This method was followed accordingly as mentioned by Jadav, 2018 [8].

### 3. Results and Discussion

Spore germination inhibition technique was used in which one drop of each chemical suspension and spore suspension was placed on a glass cavity slide, so concentration was obtained as per require for evaluation. The slides were placed in petri plates lined with moistened coarse filter paper to provide sufficient humidity for germination of conidia for 24 hours at room temperature. After 24 hours, 48 hours and 72 hours intervals observation of spore germination inhibition was taken by using light microscope at 40x objective lens. The observations on the spore germination inhibition of the fungus in each treatment including check were taken and per cent inhibition was calculated on the basis of the difference between inhibition obtained in the treatment and control. This method was followed accordingly as mentioned by Jadav, 2018 [8].

### 3.1 In vitro evaluation on efficacy of non-systemic fungicides on spore germination inhibition of *E. polygoni* of fenugreek

To check the efficacy of non-systemic fungicides, six fungicides were tested using modified slide spore germination technique on *E. polygoni*. Data on spore germination inhibition are given in Table 1.

It was clear from the data presented in Table 1 that all the fungicides significantly increased cumulative spore germination inhibition of *E. polygoni* after the 24 hrs, 48 hrs and 72 hrs. The spore germination inhibition was found to be increased with increasing the concentration of fungicides. Among the non-systemic fungicide, significantly the maximum (70.65%) mean spore germination inhibition was recorded in wettable sulphur 80% WP followed by mancozeb 75% WP with 64.28% per cent inhibition. The next effective treatment was chlorothalonil 75% WP, with 60.71 per cent inhibition. Captan 75% WP and metiram 70% WDG was found as moderately effective fungicides with 41.63% and 35.71% per cent mean inhibition, respectively. The lowest per cent mean spore germination inhibition of 24.25% per cent was recorded in treatment of zineb 75% WP at 24 hrs. The minimum spore germination was recorded in zineb 75% WP at 0.10 per cent concentration with 15.40 per cent followed by at 0.15 (19.55%) per cent.

The spore germination inhibition was increased at 48 hrs after the treatment. The data showed that significantly highest mean spore germination inhibition was recorded in treatment of wettable sulphur 80% WP (74.86%) followed by mancozeb 75% WP with 67.63% per cent. The next effective treatment was chlorothalonil 75% WP at 64.16 per cent inhibition. The moderate effective treatments were captan 75% WP and metiram 70% WDG with 46.61 and 39.41 per cent inhibition, respectively. But the less effective fungicide was zineb% WP with 27.46 per cent mean spore germination inhibition. Data were also observed that with increasing concentration of all fungicides, growth inhibition of pathogen were also increased. It revealed that mancozeb at 0.15 per cent (66.31%) was at par with its higher concentration 0.20 per cent (68.17%). Other concentrations were statically differed with each other.

### Table 1: Effect of non-systemic fungicides on spore germination inhibition of *E. polygoni* in vitro

| Sr. No. | Fungicide      | Conc. (%) | 24 hrs | 48 hrs | 72 hrs |
|---------|----------------|-----------|--------|--------|--------|
| 1.      | Mancozeb 75% WP| 0.10      | 49.79  | 51.45  | 53.30  |
|         |                | 0.15      | 52.67  | 54.52  | 56.63  |
|         |                | 0.20      | 53.68  | 55.66  | 58.06  |

Where

\[ \text{I} = \frac{C - T}{C} \times 100 \]

- **I** = Per cent inhibition
- **C** = Number of germinated spores in control.
- **T** = Number of germinated spores in treatment

2.2 Evaluation of different fungicides

Six non-systemic fungicides viz., chlorothalonil 75% WP, mancozeb 75% WP, zineb 75% WP, captan 75% WP, metiram 70% WDG and wettable sulphur 80% WP with four concentrations (0.10, 0.15, 0.20 and 0.25 per cent), six systemic fungicides viz., carbenadiazim 50% WP, tebuconazole 25% EC, picoxyorobin 22.52% SC, fusilazole 40% EC, hexaconazole 5% SC and propiconazole 25% EC with four concentrations (0.005, 0.010, 0.025 and 0.05 per cent) and six ready mix fungicides viz., hexaconazole 5% + validamycin 2.5% SC, tebuconazole 10% + sulphur 65% WG, pyraclostrobin 13.3% + epoxiconazole 5% SE, tebuconazole 50% + trifloxystrobin 25% WG, azoxystrobin 11% + tebuconazole 18.3% SC and azoxystrobin 7.1% + propiconazole 11.9% SE with four concentrations (0.005, 0.010, 0.025 and 0.05 per cent) were evaluated against test pathogen under laboratory condition by following spore germination inhibition technique. Experiment was laid out with six treatments and each treatment repeated three times. Completely Randomized block Design with Factorial Concept was used for analyzing the data.

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The significantly highest (78.80%) mean spore germination inhibition was obtained in the treatment of wettable sulphur 80% WP at 72 hrs. The next effective treatments were mancozeb 75% WP and chlorothalonil 75% WP with 71.31 and 67.85 per cent mean spore germination inhibition, respectively. Moderately effective fungicides were captan 75% WP and metiram 70% WDG with 49.97 and 42.68 per cent mean spore germination inhibition, respectively. Least effective fungicide was zineb 75% WP at with 33.14 per cent mean spore germination inhibition.

Among the non-systemic group, sulphur fungicide was reported as highly effective as compared to other fungicide for spore germination inhibition of *E. polygoni*. Related to mode of action of sulphur compound in the fungi, different researchers have different view on it. At first it was observed that sulphur could not be the toxic agent in plant but it is toxic to fungi and reduce their mycelial growth (Nane and Thapliyal, 1993) [12].

The present findings were in correspondence to the following scientists. The effectiveness of wettable sulphur in spore germination inhibition of *E. polygoni* has been reported by various workers causing powdery mildew in pea (Tripathi *et al.*, 2001) [13], fenugreek (Chovatiya, 2010) [4], coriander (Goswami, 2016) [6], mung bean (Vekariya, 2016) [10], cumin (Khunt, 2016) [9] and okra (Jadav, 2018) [8].

### 3.2 Efficacy of systemic fungicides on spore germination inhibition *E. polygoni*

Six systemic fungicides were tested against spore germination inhibition of *E. polygoni* at concentration 0.005, 0.01, 0.025 and 0.05 per cent using glass cavity slide technique. The percent spore germination inhibition obtained statistically analyzed and presented in Table 2.

It is evident from the data presented in Table 2 that all the fungicides significantly increased cumulative spore germination inhibition of *E. polygoni* after the 24 hrs, 48 hrs and 72 hrs. The spore germination inhibition was found to be increased with increasing the concentration of fungicides. At 24 hrs after treatment, all systemic fungicides were capable to inhibiting the spore germination at all tested concentrations. The results indicated that the most effective fungicide was propiconazole 25% EC with maximum mean inhibition of 70.36 per cent. Then second effective fungicide was hexaconazole 5% SC with 68.35 mean per cent spore germination inhibition. Then minimum mean of spore germination inhibition (28.63%) showed in picoxystrobin 22.52% SC and followed by azoxystrobin 23% EC with 33.32 mean spore germination inhibition.

Another results revealed that at 48 hrs after treatment, the most effective fungicide was propiconazole 25% EC with mean maximum spore germination inhibition of 74.64 per cent followed by hexaconazole 5% SC with 73.39 per cent inhibition and were found statistically at par. The minimum mean spore germination inhibition (32.92%) were recorded in picoxystrobin 22.52% SC and followed by azoxystrobin 23% EC with 33.32 per cent spore germination inhibition.
Among the systemic group, the effectiveness of propiconazole and hexaconazole were reported highest as compared to other treatments. Triazoles group of fungicides i.e., propiconazole, hexaconazole interfere with biosynthesis of sterols i.e., inhibits the biosynthesis of ergosterol which is essential for structure of cell wall and its absence causes irreversible damage to cell wall and ultimately fungus dies. In addition, they are known to impede conidial and haustoria formation. They change the sterol content and saturation of the polar fatty acids leading to alterations in membrane permeability and behavior of membrane bound enzymes (Nane and Thapliyal, 1993) [12]. The effectiveness of propiconazole against Erysiphe pisi has been reported earlier by Biju (2000) [13]. Triazole fungicides viz., propiconazole and hexaconazole were highly effective in spore germination inhibition of E. polygoni in present investigation. The effectiveness of triazole group of fungicides against powdery mildew (E. polygoni) of green gram (Venkatrao, 1997, Khunti et al., 2005) [17, 18], powdery mildew (E. polygoni) of fenugreek (Chovatiya, 2010) [4], powdery mildew (E. polygoni) of cumin (Pipliwal, 2013) [19], powdery mildew (E. polygoni) of cumin (Khunt, 2016) [9], and powdery mildew (E. polygoni) of coriander (Goswami, 2016) [10] has been reported by various workers.

### Table 2: Effect of systemic fungicides on spore germination inhibition of E. polygoni in vitro

| Sr. No. | Fungicide             | Conc. (%) | 24 hrs | 48 hrs | 72 hrs |
|---------|-----------------------|-----------|--------|--------|--------|
| 1.      | Propiconazole 25% EC  | 0.005     | 49.69 (58.15)* | 51.41 (61.10) | 53.94 (65.35) |
|         |                       | 0.010     | 55.34 (67.66) | 57.38 (70.94) | 60.62 (75.93) |
|         |                       | 0.025     | 60.05 (75.07) | 62.28 (78.37) | 64.46 (81.42) |
|         |                       | 0.050     | 63.84 (80.56) | 66.56 (84.18) | 68.57 (86.65) |
| Mean    |                       |           | 57.23 (70.36) | 59.41 (73.64) | 61.90 (77.34) |
| 2.      | Tebuconazole 25.9% EC | 0.005     | 32.79 (29.32) | 34.82 (32.60) | 37.37 (36.84) |
|         |                       | 0.010     | 36.73 (35.76) | 38.67 (39.04) | 42.92 (46.38) |
|         |                       | 0.025     | 41.34 (43.64) | 43.23 (46.91) | 45.66 (51.15) |
|         |                       | 0.050     | 46.36 (52.36) | 48.24 (55.64) | 52.68 (63.25) |
| Mean    |                       |           | 39.30 (40.27) | 41.24 (43.55) | 44.66 (49.41) |
| 3.      | Picoxystrobin 22.52% SC | 0.005    | 21.66 (13.62) | 24.30 (16.94) | 27.42 (21.21) |
|         |                       | 0.010     | 29.04 (23.56) | 31.21 (26.84) | 33.89 (31.09) |
|         |                       | 0.025     | 36.54 (35.45) | 38.49 (38.73) | 40.96 (42.97) |
|         |                       | 0.050     | 42.63 (45.88) | 44.52 (49.16) | 47.34 (54.08) |
| Mean    |                       |           | 35.05 (33.32) | 36.93 (36.38) | 40.14 (41.80) |
| 4.      | Azoxystrobin 23% EC   | 0.005     | 27.54 (21.37) | 29.20 (23.80) | 31.12 (26.72) |
|         |                       | 0.010     | 32.13 (28.29) | 34.19 (31.57) | 36.76 (35.81) |
|         |                       | 0.025     | 37.91 (37.75) | 39.83 (41.03) | 43.44 (47.27) |
|         |                       | 0.050     | 42.62 (45.85) | 44.50 (49.13) | 49.25 (57.38) |
| Mean    |                       |           | 35.05 (33.32) | 36.93 (36.38) | 40.14 (41.80) |
| 5.      | Fusilazole 40% EC     | 0.005     | 36.87 (36.00) | 38.81 (39.28) | 41.85 (44.51) |
|         |                       | 0.010     | 41.22 (43.42) | 43.11 (46.70) | 45.54 (50.94) |
|         |                       | 0.025     | 42.39 (45.45) | 44.27 (48.73) | 46.70 (52.97) |
|         |                       | 0.050     | 45.31 (50.55) | 46.62 (52.83) | 50.42 (59.41) |
| Mean    |                       |           | 41.45 (43.86) | 43.20 (46.89) | 46.13 (51.96) |
| 6.      | Hexaconazole 5% SC    | 0.005     | 52.50 (62.93) | 54.46 (66.22) | 57.08 (70.46) |
|         |                       | 0.010     | 54.73 (66.66) | 56.97 (70.29) | 60.13 (75.20) |
|         |                       | 0.025     | 57.07 (70.45) | 61.76 (77.61) | 63.21 (79.68) |
|         |                       | 0.050     | 58.92 (73.35) | 63.04 (79.45) | 65.06 (82.23) |
| Mean    |                       |           | 55.80 (68.35) | 59.06 (73.39) | 61.37 (76.89) |

F= Fungicide, C=Concentration, F*C=Interaction of fungicide and concentration
* = Mean of three replications

*Figures in parentheses indicate arc-sine re-transformed values.

### 3.3 Efficacy of ready-mix fungicides on spore germination inhibition of E. polygoni

For testing the efficacy of ready-mix fungicides, the slide spore germination technique was used. The different ready-mix fungicides were tested under in vitro condition. The cumulative per cent inhibition of spore germination recorded for given concentrations and period i.e., 24, 48 and 72 hours are presented in Table 3.

At 24 hrs after treatment, all fungicides were capable to inhibit the spore germination at all concentrations. The results indicated that the most effective fungicide was tebuconazole 50% + trifloxystrobin 25% WG with maximum mean inhibition of 62.55 per cent followed by tebuconazole 10% + sulphur 65% WG with 60.15 per cent. The minimum mean of spore germination inhibition (43.98%) was recorded in hexaconazole 5% + validamycin 2.5% SC and followed by azyxistrobin 7.1% + propiconazole 11.9% SE with 51.40 per cent. The minimum spore germination inhibition of 34 per cent was recorded in hexaconazole 5% + validamycin 2.5% SC at 0.005 per cent concentration followed by 0.01 per cent with 38.81 per cent inhibition. Within the fungicides result found non-significant. Interaction of fungicide and concentration was found non-significant. Results revealed that at 48 hrs after treatment, were most effective fungicide was tebuconazole 50% + trifloxystrobin
25% WG with maximum mean spore germination inhibition of 65.67 per cent followed by tebuconazole 10% + sulphur 65% WG with 62.39 per cent mean inhibition. But the least mean spore germination inhibition (46.93%) were recorded in hexaconazole 5% + validamycin 2.5% SC followed by azoxystrobin 7.1% + propiconazole 11.9% SE with 55.63 per cent.

The result of 72 hrs treatment showed that most effective fungicide was tebuconazole 50% + trifloxystrobin 25% WG with maximum mean spore germination inhibition of 70.10 per cent followed by tebuconazole 10% + sulphur 65% WG with 66.57 per cent. The minimum mean spore germination inhibition 50.97 per cent was recorded in hexaconazole 5% + validamycin 2.5% SC.

The effectiveness of ready-mix fungicides among the six fungicides tebuconazole 50% + trifloxystrobin 25% WG was recorded highest spore germination inhibition at their higher concentration. The above result showed that the per cent spore germination inhibition in all the treatments were somewhat increased with increasing in period and concentration. This ready-

| Sr. No. | Fungicide | Conc. (%) | Per cent spore germination inhibition after a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z,a,b,c | 24 hrs | 48 hrs | 72 hrs |
|---------|------------|-----------|----------------------------------------------------------------------------------|--------|--------|--------|
| 1.      | Hexaconazole 5% + Validamycin 2.5% SC | 0.005 | 35.67 (34.00)* | 37.44 (36.95) | 39.93 (41.19) |
|         |            | 0.010 | 38.53 (38.81) | 40.26 (41.76) | 42.54 (45.72) |
|         |            | 0.025 | 42.66 (45.92) | 43.46 (48.88) | 46.63 (52.84) |
|         |            | 0.050 | 49.13 (57.19) | 50.85 (60.14) | 53.82 (65.15) |
| Mean    |            | 41.50 (43.98) | 43.23 (46.93) | 45.73 (50.97) |
| 2.      | Tebuconazole 10% + Sulphur 65% WG | 0.005 | 41.83 (44.48) | 43.53 (47.43) | 45.80 (51.39) |
|         |            | 0.010 | 48.28 (55.72) | 49.20 (57.30) | 51.30 (60.91) |
|         |            | 0.025 | 54.21 (65.80) | 55.38 (67.73) | 58.13 (72.12) |
|         |            | 0.050 | 59.73 (74.59) | 61.41 (77.09) | 64.80 (81.87) |
| Mean    |            | 51.25 (60.15) | 52.38 (62.39) | 55.01 (66.57) |
| 3.      | Pyraclostrobin 13.3% WP + Epoxiconazole 5% WP | 0.005 | 42.65 (45.91) | 43.40 (47.21) | 45.67 (51.17) |
|         |            | 0.010 | 47.11 (53.68) | 48.04 (55.29) | 50.33 (59.25) |
|         |            | 0.025 | 49.55 (57.90) | 51.87 (61.87) | 53.82 (65.15) |
|         |            | 0.050 | 53.63 (64.83) | 58.86 (73.29) | 61.41 (77.09) |
| Mean    |            | 48.47 (56.08) | 51.18 (59.42) | 52.18 (62.17) |
| 4.      | Tebuconazole 50% EC + Trifloxystrobin 25% WG | 0.005 | 46.74 (53.04) | 48.83 (56.66) | 50.16 (58.96) |
|         |            | 0.010 | 50.24 (59.09) | 52.95 (63.70) | 55.34 (67.65) |
|         |            | 0.025 | 55.24 (67.50) | 56.02 (68.77) | 58.59 (72.83) |
|         |            | 0.050 | 57.17 (70.60) | 59.05 (73.56) | 64.14 (80.97) |
| Mean    |            | 52.35 (62.55) | 54.21 (65.67) | 57.06 (70.10) |
| 5.      | Azoxystrobin 11% + Tebuconazole 18.3% SC | 0.005 | 40.29 (41.81) | 41.99 (44.76) | 44.27 (48.72) |
|         |            | 0.010 | 44.58 (49.26) | 46.27 (52.21) | 47.97 (55.17) |
|         |            | 0.025 | 48.17 (55.52) | 50.08 (58.81) | 52.20 (62.43) |
|         |            | 0.050 | 54.80 (66.78) | 56.62 (69.73) | 60.32 (75.48) |
| Mean    |            | 46.96 (53.34) | 48.74 (56.38) | 51.19 (60.45) |
| 6.      | Azoxystrobin 7.1% + Propiconazole 11.9% SE | 0.005 | 39.05 (39.68) | 42.53 (45.69) | 43.65 (47.65) |
|         |            | 0.010 | 42.21 (45.15) | 43.91 (48.10) | 46.18 (52.06) |
|         |            | 0.025 | 47.87 (55.01) | 50.58 (59.67) | 51.90 (61.93) |
|         |            | 0.050 | 54.18 (65.75) | 56.20 (69.05) | 59.88 (74.81) |
| Mean    |            | 45.83 (51.40) | 48.30 (55.63) | 50.40 (59.11) |
| C. C. F |          | 0.56 | 0.46 | 1.12 | 0.43 | 0.35 | 0.87 | 0.74 | 0.61 | 1.49 |
| C. D. at % |         | 1.59 | 1.30 | NS | 1.24 | 1.01 | 2.47 | 2.11 | 1.72 | NS |
| C. V. % |          | 4.07 | 3.03 | 4.96 |

Fungicide, C=Concentration, F*C=Interaction of fungicide and concentration
* = Mean of three replications
* Figures in parentheses indicate arc-sine re-transformed values.

mix of fungicides has combined effect of triazole group and strobilurin group. Triazole group of fungicides interfere with biosynthesis of sterols and strobilurin group fungicides may inhibit electron transfer in mitochondria, disrupting metabolism and also inhibit respiratory chain (Nane and Thapliyal, 1993) [12]. The effectiveness of tebuconazole + trifloxystrobin has been reported to manage powdery mildew (E. polygoni) of cumin (Khunt, 2016) [9], powdery mildew (E. polygoni) of coriander (Goswami, 2016) [6] and powdery mildew (E. cichoracearum) of okra (Jadav, 2018) [8].

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