International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 29-34
© 2018 IJCS
Received: 18-01-2018
Accepted: 19-02-2018

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Evaluation of different fungicides against fenugreek wilt (*Fusarium oxysporum* Schlecht.)

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Abstract
Fenugreek is an annual forage legume and spice crop and is widely known for its medicinal, pharmaceutical and nutraceutical properties. It is prone to attack by several diseases. A severe outbreak of wilt of fenugreek was observed causing considerable damage in the field of Saurashtra region. Experiment was conducted to find out effective fungicides for the management of fenugreek wilt. Different fungicides were tested against *Fusarium oxysporum* Schlecht. *viz.,* systemic, non-systemic and fungicide combination. In laboratory screening of different Agro-chemicals, tebuconazole 25.9% EC was found to be quite effective in inhibiting the radial growth of test pathogen among systemic group of fungicides, while in non-systemic group of fungicides, copper oxychloride 50% WP and copper hydroxide 77% WP, in case of fungicide combinations tebuconazole 50% + trifloxystobin 25% WG were significantly inhibited the growth of test fungus *in vitro*. Fungicides found most effective *in vitro* were further tested in net house condition in pot culture. Among six fungicides tested, the combination of carbendazim 12% + mancozeb 63% WP showed minimum PDI followed by carbendazim 50% WP.

Keywords: fungicide, fenugreek, wilt, *Fusarium oxysporum* schlecht

1. Introduction
Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage legume and spice crop and is widely known for its medicinal, pharmaceutical and nutraceutical properties. Fenugreek is believed to be native to the Mediterranean region (Petropoulos, 2002) [20], but now is grown as a spice in most parts of the world. It is reported as a cultivated crop in parts of Europe, northern Africa, west and south Asia, Argentina, Canada, United States of America (USA) and Australia (Fazli and Hardman, 1968; Edison, 1995) [8, 7]. Fenugreek is regarded as the oldest known medicinal plant in recorded history (Lust, 1986) [13]. Fenugreek has been referred to as a medicinal herb both in Indian Ayurvedic and traditional Chinese medicines (Tiran, 2003) [26]. Gujarat occupies an area of 5000 hectares, with production of 14000 tonnes (Anon., 2014) [4]. There are several factors responsible for low productivity in which disease causes considerable losses. Fenugreek is suffering from various diseases like wilt, powdery mildew, downey mildew, rust, leaf spot, root rot, stem rot, anthracnose, bacterial blight. *F. oxysporum* is an abundant and active saprophyte in soil and organic matter, with some specific forms that are plant pathogenic (Smith *et al.,* 1988) [25]. Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant debris. The fungus can survive either as mycelium, or as any of its three different spore types (Agrios, 2005) [2]. *F. oxysporum* can survive as mycelium and chlamydospores in seed and soil, and also on infected crop residues, roots and stem tissue buried in the soil for more than six years (Singh *et al.,* 2007) [23]. Chlamydospores can survive in soil either in dormant form or saprophytically without a suitable host. The fungus can also advance laterally as the mycelium penetrates the adjacent xylem vessels through the xylem pits (Agrios, 2005) [2].

2. Materials and Methods
2.1. Isolation and purification of pathogen
Fenugreek plants, naturally infected and showing typical wilt symptoms were collected from different fifteen locations of Saurashtra regions of Gujarat and brought to the laboratory. Isolation of the fungus was made by tissue isolation technique on potato dextrose agar (PDA) and incubated at 28 ± 2^0 C. The resulting fungal culture was purified by hyphal tip method. The fungus was isolated, purified and sub cultured in aseptic condition. The isolates of the pathogen were identified based on colony characters and spores morphology (Booth, 1971) [6].

The fifteen isolates were screened for their pathogencity on fenugreek cultivar Gujarat Methi-2 during *rabi* season 2016-17 under net house. Isolate which showed highest per cent disease incidence was used for evaluation of fungicides.

2.2. *In vitro* evaluation of different fungicides against test pathogen

In vitro efficacy of different fungicide against fenugreek wilt pathogen was evaluated by poisoned food technique (Nene and Thapliyal, 1993) [17] as described below:

2.2.1. Poisoned food technique

Required quantity of fungicide was added in 100 ml of luck warm PDA media and mixed thoroughly. This solution was poured into Petri plates about 20 ml in each. After solidification of media 5 mm discs of four days old culture of test pathogen were inoculated at the center of Petri plates and then incubated at 28 ± 2°C. Three replications were maintained for each fungicide. Medium without fungicide was kept as control. Per cent inhibition of the growth of the fungus over the control was calculated by using the following formula: (Vincent, 1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent reduction in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatments.

Systemic fungicides i.e., azoxystrobin 23% SC, hexaconazole 5% SC, tebuconazole 25.9% EC, thiophanate methyl 70% WP, carbendazim 50% WP and flusilazole 40% EC were used under laboratory condition at 100, 250 and 500 ppm concentration.

Non systemic fungicides i.e., captan 50% WP, thiram 75%WP, mancozeb 75% WP, propineb 70%WP, copper hydroxide 77% WP and copper oxychloride 50% WP were used at 2000, 2500 and 3000 ppm concentration.

Ready mix fungicides i.e., zineb 68% + hexaconazole 4% WP, carbendazim 12% + mancozeb 63% WP, azoxystrobin 11% + tebuconazole 18.30% SC, carboxin 37.5% + thiram 37.5% WS, tebuconazole 50% + trifloxystrobin 25% WG, cymoxanil 8% + mancozeb 64% WP were evaluated at 250, 500 and 1000 ppm concentration under laboratory condition against fenugreek wilt pathogen following poisoned food technique as described earlier. 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.

2.3. Evaluation of fungicides in pot condition

Fungicides which gave higher per cent inhibition in growth of test pathogen during laboratory tests were evaluated in pots under net house condition during *rabi* season 2016-17.

2.3.1. Mass multiplication of *Fusarium oxysporum* wilt of fenugreek

The test pathogen *Fusarium oxysporum* was mass multiplied on sterilized sorghum grains for pot culture studies. For this, 100 g of sorghum grains were washed thoroughly in tap water and boiled. After that removing the excess water, grains were allowed to air dry and cooled at room temperature. Polythylene bags were filled up with about 200 grams of grains. Mouth of these bags was packed with piece of PVC pipe and non absorbant cotton. Than it autoclaved at 121 °C for 20 min at 15 and inoculated 4 days old culture of test pathogen. After seven days the inoculum was mixed with sterilized soil in pots @ 40 g kg^{-1}.

2.3.2 Pot filling and inoculation of mass multiplied cultures

Sterilized soil was used for pot filling. About 40 g inoculum was added per kg soil. Sterilized soil and inoculums was thoroughly mixed in pot. For each fungicide three sets of pots (20 cm width x 20 cm depth) were prepared. One set of pot constituting three pots were considered as inoculated control. Three test tubes fill with soil were inserted at equidistance and about 6 cm deep in each pot for secondary inoculation. Secondary inoculation done (when drenched and give cfu/ml and quantity in ml) by inoculum prepared on potato dextrose broth. It was done after three weeks of sowing by removing test tubes. It was done by pouring about 30 ml liquid culture per pot along with mycelial met in hole made by removal of test tubes so that inoculum was directly leached to the root zone.

2.3.3 Drenching of fungicides and its effect on wilt disease

Two fungicides from each group i.e., systemic, non-systemic and combination which was superior in growth inhibition of test pathogen *in vitro* was used for pot culture studies. Fungicides used in pot culture studies were tebuconazole 25.9% EC (0.05%), carbendazim 50% WP (0.05%), copper oxychloried 50% WP (0.2%), captan 50% WP (0.3%), carbendazim 12% WP + mancozeb 63% WP (0.1%), tebuconazole 50% + trifloxystrobin 25% WG (0.1%).

Drenching of selected fungicides was made at about 30 DAS. Three replications were maintained for each fungicide concentration. One set of pot constituting three pots were maintained as inoculated control which was not drenched with any chemical.

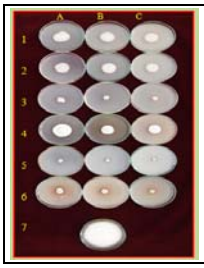
$$\text{Per cent disease incidence} = \frac{\text{Number of wilted plants per pot}}{\text{Total number of plants per pot}} \text{ x100}$$

3. Results and Discussion

3.1 *In vitro* evaluation of systemic fungicides against *Fusarium oxysporum*

Efficacy of six commonly used systemic fungicides *viz*., hexaconazole, azoxystrobin, flusilazole, thiophanate methyl, tebuconazole, and carbendazim were evaluated against *F. oxysporum* wilt of fenugreek at different concentrations *viz*., 100, 250 and 500 ppm using poisoned food technique as described in section 2.2.1. The data revealed that all the fungicides at all concentrations reduced mycelial growth (Table 2) of *F. oxysporum* as compared to control (Fig. - 1).

It is evident from the data presented in Table 2 and Fig.- 1, that maximum mean per cent inhibition of *F. oxysporum* mycelial growth was recorded in tebuconazole (88.96%) followed by carbendazim (83.23%), flusilazole (82.71%), azoxystrobin (62.67%), thiophanate methyl (61.10%), and hexaconazole (60.67%). hexaconazole was found to be least effective against *F. oxysporum* at 250 and 500 ppm (Fig. - 1).



Sr. No.	Systemic fungicides	Concentration (ppm)
1.	Hexaconazole 5% SC	A= 100
2.	Azoxystrobin 23% SC	
3.	Flusilazole 40 % EC	B = 250
4.	Thiophanate methyl 70% WP	
5.	Tebuconazole 25.9% EC	C = 500
6.	Carbendazim 50% WP	
7.	Control	

Fig 1: Radial growth of *Fusarium oxysporum* on PDA as influence by different systemic fungicides at various concentration *in vitro*

Table 1: *In vitro* evaluation of systemic fungicides against *Fusarium oxysporum*

Fungicides	Growth inhibition (%)			Mean inhibition (%)
	100 ppm	250 ppm	500 ppm	
Hexaconazole 5% SC	48.82* (56.63)	51.14 (60.63)	53.58 (64.75)	51.18 (60.67)
Azoxystrobin 23% SC	50.58 (59.69)	51.25 (60.83)	55.24 (67.5)	52.35 (62.67)
Flusilazole 40 % EC	62.99 (79.38)	65.50 (82.81)	67.98 (85.94)	65.49 (82.71)
Thiophanate methyl 70% WP	44.64 (49.38)	54.11 (65.63)	55.74 (68.31)	51.49 (61.10)
Tebuconazole 25.9% EC	69.03 (87.19)	70.40 (88.75)	72.49 (90.94)	70.64 (88.96)
Carbendazim 50% WP	63.89 (80.63)	65.50 (82.81)	68.23 (86.25)	65.87 (83.23)
Mean	56.66 (68.82)	59.65 (73.58)	62.21 (77.28)	59.50 (73.22)
	Fungicide(F)		Concentration(C)	
S.Em. ±	0.18		0.12	
C.D at 5%	0.52		0.36	
CV%	0.92			

* Data outside the parenthesis are arcsine transformed whereas inside are re-transformed values.

There was found positive correlation between concentration and inhibition of growth of pathogen. It is also observed that with increasing concentration of all fungicides, inhibition of growth of pathogen also increased. The tebuconazole (500 ppm), carbendazim (500 ppm), flusilazole (500 ppm), azoxystrobin (500 ppm), thiophanate methyl (500 ppm), and hexaconazole (500 ppm) showed 88.96, 83.23, 82.71s, 62.67, 61.10 and 60.67 per cent inhibition of mycelial growth, respectively which are higher than their lower concentration of 100 and 250 ppm (Fig - 1). \

These results are similar to the evaluation of systemic fungicides carried out by Mailem *et al*. (2015) [15]. They observed that carbendazim (0.1%) and tebuconazole (0.1%) were found superior which completely inhibited growth of *F. oxysporum* f. sp. *ciceri*. Whereas Ahmad and Abu, (1989) [3], Gupta *et al*. (1997) [10], Singh *et al*. (2003) [24], Khokhar, (2012) [11] achieved good control of *F. oxysporum* with carbendazim in different crops.

Looking to concentration of individual fungicides, all the fungicides differed significantly in inhibition of test pathogen i.e. 100, 250 and 500 ppm.

3.2 *In vitro* evaluation of non-systemic fungicides against *Fusarium oxysporum*

Efficacy of six commonly used non-systemic fungicides *viz*., propineb, copper hydroxide, captan, copper oxychloride, thiram and mancozeb were evaluated against *Fusarium oxysporum* at different concentrations *viz*., 2000, 2500 and 3000 ppm using poisoned food technique as described in section 2.2.1. The data revealed that all the fungicides at various concentrations reduced mycelial growth (Table 3) of *Fusarium oxysporum* as compared to control (Fig. - 2).

Sr. No.	Non systemic fungicides	Concentration (ppm)
1.	Propineb 70%WP	A= 2000
2.	Copper hydroxide 77% WP	
3.	Captan 50% WP	B = 2500
4.	Copper oxychloride 50% WP	
5.	Thiram 75%WP	C = 3000
6.	Mancozeb 75% WP	
7.	Control	

Fig 2: Radial growth of *Fusarium oxysporum* on PDA as influence by different non systemic fungicides at various concentration *in vitro*

It is inferred from the data presented in Table 3 and Fig. -2 that all the fungicides are significantly effective in inhibition of test pathogen.

Table 2: *In vitro* evaluation of non-systemic fungicides against *Fusarium oxysporum*

Fungicides	Growth inhibition (%)			Mean inhibition (%)
	2000 ppm	2500ppm	3000 ppm	
Propineb 70%WP	23.77* (16.25)	30.00 (25.00)	38.50 (38.75)	30.75 (26.66)
Copper hydroxide77% WP	89.19 (99.98)	89.19 (99.98)	89.19 (99.98)	89.19 (99.98)
Captan 50% WP	46.43 (52.50)	48.95 (56.88)	52.24 (62.50)	49.20 (57.29)
Copper oxychloride 50% WP	89.19 (99.98)	89.19 (99.98)	89.19 (99.98)	89.19 (99.98)
Thiram 75%WP	45.72 (51.25)	48.59 (56.25)	51.50 (61.25)	48.60 (56.25)
Mancozeb 75% WP	23.77 (16.25)	33.99 (31.25)	37.76 (37.50)	31.84 (28.33)
Mean	53.01 (56.03)	56.65 (61.56)	59.73 (66.66)	56.46 (61.41)
	Fungicide(F)		Concentration(C)	
S.Em. ±	0.09		0.06	
C.D at 5%	0.26		0.19	
CV%	0.50			

* Data outside the parenthesis are arcsine transformed whereas inside are re-transformed values.

The perusal of data presented in Table 3 and Fig. -2 revealed that mean per cent growth inhibition of *Fusarium oxysporum* mycelial growth was cent per cent in copper oxychloride and copper hydroxide (99.98%) which was followed by captan (57.29%), thiram (56.25%) and mancozeb (28.33%).They are significantly differed to each other.

There was found positive correlation between concentration and inhibition of growth of pathogen. It is also observed that with increasing concentration of all fungicides, inhibition of growth of pathogen also increased except copper oxychloride (2000 ppm) and copper hydroxide (2000 ppm) which give almost cent per cent inhibition at lower concentration. The fungicides propineb, captan, thiram and mancozeb showed 26.66, 57.29, 56.25, and 28.33 per cent inhibition of mycelial growth, respectively which are higher than their lower concentration of 2000 and 2500 ppm (Fig - 2).

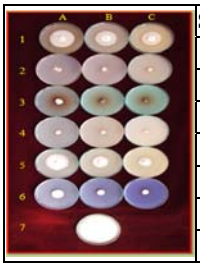
Vasoya (1993) [27] reported that non systemic fungicides copper oxycloride was good inhibitor of test pathogen *Fusarium oxysporum* f. sp. *ciceri* even at low concentration i.e. 1000 and 1500 ppm to the extent of 88.35 and 90.03 per cent whereas cent per cent inhibition was found with 2000, 2500 and 3000 ppm.

Parmar (2014) [18] reported that non systemic fungicide copper hydroxide was found effective and inhibited 75.30 per cent growth of test pathogen *Fusarium oxysporum* f. sp. *niveum.* The effectiveness of thiram against *Fusarium oxysporum* has been recorded by Sharma and Jain (1984) [21], Gupta and Bansal (2003) [9] and Barhate *et al*. (2015) [5].

Looking to concentration of individual fungicides, all the fungicides differed significantly in inhibition of test pathogen.

3.3 *In vitro* evaluation of fungicide combinations against *Fusarium oxysporum*

The results of the laboratory evaluation of ready mix fungicides on the radial growth of *F. oxysporum* wilt of fenugreek are presented in the Table 4 and Fig. - 3. The results of the experiment showed that all selected fungicide combinations at different levels inhibited the radial growth of tested pathogen.



Sr. No.	Fungicide combinations	Concentration (ppm)
1.	Zineb 68% + Hexaconazole 4% WP	A= 250
2.	Tebuconazole 50% + Trifloxystobin 25% WG	
3.	Carbendazim 12%+ Mancozeb 63% WP	B = 500
4.	Azoxystrobin 11% + Tebuconazole 18.30% SC	
5.	Cymoxanil 8% + Mancozeb 64% WP	C = 1000
6.	Carboxin 37.5% + Thiram 37.5% SD	
7.	Control	

Fig 3: Radial growth of *Fusarium oxysporum* on PDA as influence by different fungicide combinations at various concentration *in vitro*

It is evident from the data presented in Table 4 and Fig. - 3, all the fungicides were significantly effective in inhibiting growth of pathogen. The maximum growth inhibition 84.94 per cent was recorded in fungicide combination, tebuconazole 50% + trifloxystobin 25% WG which was closely followed by azoxystrobin 11% + tebuconazole 18.30% SC (84.75%) and carbendazim 12% + mancozeb 63% WP (84.28%). Fungicide combinations carboxin 37.5% + thiram 37.5% WP, cymoxanil 8% + mancozeb 64% WP and zineb 68% + hexaconazole 4% WP gave 77.84,56.63 and 46.59% inhibition, respectively. The maximum growth inhibition of 87.50 per cent was recorded in fungicide combinations carbendazim 12%+ mancozeb 63% WP at 3000 ppm concentarion (Fig. -3). They were statistically differed to each other.

Table 3: *In vitro* evaluation of fungicide combinations against *Fusarium oxysporum*

Fungicides	Growth inhibition (%)			Mean inhibition (%)
	250 ppm	500 ppm	1000 ppm	
Zineb 68% + Hexaconazole 4% WP	38.76* (39.20)	43.69 (47.73)	46.63 (52.84)	43.02 (46.59)
Tebuconazole 50% + Trifloxystobin 25% WG	65.84 (83.24)	67.41 (85.23)	68.33 (86.36)	67.19 (84.94)
Carbendazim 12%+ Mancozeb 63% WP	63.31 (79.83)	67.63 (85.51)	69.30 (87.50)	66.74 (84.28)
Azoxystrobin 11% + Tebuconazole 18.30% SC	64.98 (82.10)	67.4 (85.23)	68.82 (86.93)	67.06 (84.75)
Cymoxanil 8% + Mancozeb 64% WP	40.42 (42.05)	50.90 (60.23)	55.31 (67.61)	48.87 (56.63)
Carboxin 37.5% + Thiram 37.5% WS	56.72 (69.89)	63.93 (80.68)	65.61 (82.95)	62.08 (77.84)
Mean	55.00 (60.44)	60.05 (74.10)	59.76 (77.36)	59.16 (72.50)
	Fungicide(F)		Concentration(C)	
S.Em. ±	0.25		0.18	
C.D at 5%	0.73		0.51	
CV%	1.29			

* Data outside the parenthesis are arcsine transformed whereas inside are re-transformed values.

Patra and Biswas (2016) reported tebuconazole 50% + trifloxystobin 25% WG as effective fungicides against *F. oxysporum* f. sp. *ciceri*. Whereas Kumari *et al.* (2014) [12] and Barhate *et al*. (2015) [5] achieved good control of *F. oxysporum* with fungicide combination of carbendazim 12% + mancozeb 63% WP.

There was also found positive correlation between concentration and inhibition of growth of pathogen. It is also observed that with increasing concentration of all fungicides, inhibition of growth of pathogen also increased. Carbendazim 12% + mancozeb 63% WP, azoxystrobin 11% + tebuconazole 18.30% SC, tebuconazole 50% + trifloxystobin 25% WG, carboxin 37.5% + thiram 37.5% WS, cymoxanil 8% + mancozeb 64% WP and zineb 68% + hexaconazole 4% WP at 1000 ppm concentration showed 87.50, 86.93, 86.36, 82.95, 67.61 and 52.84 per cent inhibition of mycelial growth, respectively which are higher as compared to their lower concentration of 250 and 500 ppm (Fig. - 3).

3.4 Evaluation of fungicides under pot condition.

Fungicides which gave higher per cent inhibition in growth of test pathogen were evaluated in pots under greenhouse condition. Two superior fungicides were selected from each group i.e. systemic, non-systemic and fungicide combination (Table 5). Test pathogen *Fusarium oxysporum* was mass multiplied on solid medium and pot filling was done as described in section 2.3.3. Total six fungicides were drenched after 30 DAS. Each fungicide replicated thrice and one inoculated control was maintained.

Table 4: Evaluation of fungicides in pot culture

Sr. No.	Fungicide	Concentration (%)	Per cent disease incidence (%)	Per cent increase over control
1	Control	-	75.03 (93.33)	-
2	Tebuconazole 25.9% EC	0.05	40.52 (42.22)*	45.99 (54.76)
3	Carbendazim 50% WP	0.05	26.36 (20.00)	64.87 (78.57)
4	Copper oxychloride 50% WP	0.2	29.58 (24.44)	60.57 (73.80)
5	Captan 50% WP	0.3	48.20 (55.55)	35.76 (40.47)
6	Tebuconazole 50% + Trifloxystobin 25% WG	0.1	35.20 (33.33)	53.09 (64.28)
7	Carbendazim 12% + Mancozeb 63% WP	0.1	24.85 (17.77)	66.88 (80.95)
S.E.m ±			1.90	
C.D at 5%			5.88	
C.V %			9.69	

* Data outside the parenthesis are arcsine transformed whereas inside are re-transformed values.

Among fungicide tested, carbendazim 12% + mancozeb 63% WP showed minimum disease intensity (17.77%) at 0.1 per cent concentration closely followed by carbendazim 50% WP with 20.00 % disease intensity at 0.05 per cent concentration. They were at par. Copper oxychloride 50% WP at 0.2%, tebuconazole 50% + trifloxystobin 25% WG at 0.1 %, tebuconazole 25.9% EC at 0.05% and captan 50% WP at 0.3 % concentration gave 24.44, 33.33, 42.22 and 55.55 per cent growth inhibition against test pathogen. Maximum per cent increase over control was found in carbendazim + mancozeb (80.95) followed by carbendazim (78.57).

The effectiveness of carbendazim 50 WP and tebuconazole 50% + trifloxystobin 25% WG against wilt disease has been reported by Maitlo *et al.* (2014) [16] and Sharma (2011) [22].

Mahmood *et al*. (2015) [14] achieved good control of *F. oxysporum* with carbendazim.

Gupta and Bansal (2003) [9] reported carbendazim, mancozeb, captan, thiram, and thiophanate methyl at 0.2% concentration found significantly effective against *F. oxysporum* inducing fenugreek wilt under pot conditions.

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