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Evaluation of different fungicides *in vitro* against *Phomopsis vexans* causing stem blight and fruit rot in brinjal

MR Thesiya, KB Rakholiya, KN Panara and DA Bhagariya

Abstract
The Brinjal (*Solanuum melongena* L.) is principal vegetable crops grown in the tropical and subtropical areas. Stem blight and fruit rot caused by *Phomopsis vexans* (Sacc. and Syd.) Harter, is an important disease of brinjal inflicting heavy losses. It has been reported that *P. vexans* reduces yield up to 20% and marketable value of the crop nearly 20 to 30%. Therefore, in present studies 19 fungicides tested *in vitro* at three concentrations by poisoned food technique for evaluating their efficacy against *P. vexans*. Among systemic fungicides at 500 ppm significantly highest average mycelial growth inhibition was with carbendazim and propiconazole recorded i.e. 92.51% followed by hexaconazole (57.49%) and tebuconazole (56.57%), thiophanate methyl (52.08%) and azoxystrobin (45.83%). Among non-systemic fungicides at 2500 ppm significantly highest average mycelial growth inhibition was with mancozeb, propineb and chlorothalonil recorded i.e. 92.47% followed by zineb (69.58%), thiram (53.81%), copper oxychloride (49.13%) and Sulphur (40.88%). Among ready mix fungicides 2000 ppm significantly highest average mycelial growth inhibition was recorded in carboxin 37.5% + thiram 37.5%, carbendazim 12% + mancozeb 63% and pyraclostrobin 12.5% + epoxiconazole 4.7% i.e. 92.49% followed by pyraclostrobin 5% + mitiram 55% (72.96%), captan 70 % + hexaconazole 5 % (53.50%) and hexaconazole 4% + zineb 68% (38.80 %).

Keywords: Brinjal, fungicides, *Phomopsis vexans*, stem blight and fruit rot, *In vitro*, poison food technique

Introduction
The brinjal (*Solanum melongena* L.) is "King of Vegetables" also known as aubergine or egg plant from the plant family Solanaceae is an important vegetable crop grown in India as well as in other parts of the world. The major brinjal growing countries are China, India, and United State, Egypt, Indonesia, Japan and Turkey. South Asia accounts for almost 50 per cent of world brinjal area under cultivation (Harish *et al.*, 2011) [6]. In India, brinjal is mainly grown in Maharashtra, Punjab, Haryana, Gujarat, West Bengal, Karnataka, Andhra Pradesh, Madhya Pradesh, Kerala and Uttar Pradesh with an area of 735.0 thousand hectare and production was 12987.0 thousand MT of fruits with productivity of 17.7 MT/ha. (Anonymous, 2019) [3]. In Gujarat, it's grown in an area over 74.06 thousand hectare with a total production of 1471.16 thousand MT having productivity of 19.87 t/ha. (Anonymous, 2017) [2]. This crop is suffered by many diseases caused by various microbes. Among them, stem blight and fruit rot of eggplant caused by *Phomopsis vexans* (Sacc. & Syd.) Harter, is a serious disease which attacks all above ground parts of the plant (Das, 1998) [4]. The disease was first reported from the Gujarat state in 1914 and since then from many parts of India. It has been reported that *P. vexans* reduces yield (15-20%) and marketable value of the crop nearly 20 to 30% (Jain and Bhatnagar, 1980) [7]. During last few years, a most popular variety GNRB-1 and Surati Ravaiya grown under *kharif* season was found to be severely affected by stem blight and fruit rot disease in south Gujarat region. Therefore, keeping all these aspects in view present studies was aimed to found the efficacies of different concentrations of fungicides under *in vitro* against *P. vexans* causing stem blight and fruit rot in brinjal.

Materials and Methods
Isolation of pathogens
Brinjal variety *viz.*, Surati Ravaiya and GNRB-1) showing typical stem blight and fruit rot symptoms were collected and brought to the laboratory and subjected to tissue isolation.

After 72hrs of incubation, the culture appeared initially as dull white floccose mycelium with circular to irregular shape of colony which gradually turned to dark grayish white as it grew older. Numerous black, globose to irregular pycnidia were also produced in a month old culture on potato dextrose agar (PDA) medium. The culture was further purified by single hyphal tip method and the purified culture was maintained on PDA slants for further studies. The periodical sub-culturing and multiplication were made on PDA plates to keep the culture fresh and to use throughout the investigations.

In vitro evaluation of fungicides

Efficacy of seven non-systemic fungicides, six systemic and ready mix fungicides were evaluated *in vitro* at various concentrations against *P. vexans*, applying Poisoned food technique (Nene and Thapliyal, 1993) [9] and using PDA as basal culture medium. Systemic fungicides at 50, 250 and 500 ppm while, non-systemic at 1000, 2000 and 2500 and ready mix fungicides at 500, 1500 and 2000 ppm evaluated against *P. vexans*. Based on active ingredient, requisite quantity of the fungicide was mixed in 100 ml potato dextrose agar medium in 250 ml flask and well shaken to facilitate uniform mixture of fungicides and 20 ml was poured in each sterilized Petri plates. A disc of five mm was placed in the centre of each poured plate. The discs were cut with the help of a sterilized cork borer from 10 days old culture of *P. vexans*. Inoculated Petri plates were incubated at 27±1 °C. The colony growth was measured after 24 hrs interval till the entire plate of control treatment was completely covered with mycelium. Suitable check was maintained without fungicide and inoculated with *P. vexans*. The per cent growth inhibition (PGl) over control was calculated using the following formula (Vincent, 1927) [12]:

$$\text{PGI (\%)} = \frac{C-T}{C} \times 100$$

Where,
PGI (%) = Per cent growth inhibition
C=Average diameter (mm) of mycelial colony of control plate
T=Average diameter (mm) of mycelial colony of treated plate in treated plates

Results and Discussion

Effect of different systemic fungicides on growth inhibition of *P. vexans*

Effect of these fungicides on radial growth and inhibition of test pathogen were recorded. All the treatments were replicated thrice and a suitable untreated control (without fungicide) was also maintained.

Results revealed in (Plate 1 and Table 1) that the fungicides tested significantly inhibited growth of the test fungus over untreated control. Further, it was found that per cent inhibition of the test pathogen was increased with the increase in concentration of the fungicides tested. At 50 ppm concentration, significantly highest per cent growth inhibition over control was recorded in carbendazim (70.36%) followed by propiconazole (67.94%). At 250 ppm concentration, propiconazole recorded significantly highest per cent growth inhibition (74.44%) followed by carbendazim (73.71%). At 500 ppm concentration, cent per cent growth inhibition of the pathogen recorded by carbendazim and propiconazole. Next best was hexaconazole (57.49%). There was no mycelial growth of the pathogen in carbendazim and propiconazole at 500 ppm and also significantly lesser growth at 50 and 250 ppm. Thus, both the fungicides proved the growth inhibition of *P. vexans*. Next best fungicide in order of merit was hexaconazole. While, the rest of the fungicides were comparatively medium or less effective. Azoxystrobin was found least effective at all concentrations as compared to other fungicides.

The results are also in agreement with past studied. Mohanty *et al.* (1994) [8] reported that *P. vexans* was completely inhibited by carbendazim at 0.10%. Das *et al*. (2014) [5] and Reddy (2017) [11] tested that carbendazim at 0.1% showed complete inhibition of the mycelial growth.

Effect of different non systemic fungicides on the growth of *P. vexans*

Results revealed in (Plate 2 and Table 2) that all non-systemic tested significantly inhibited mycelial growth of *P. vexans*, over untreated control. At 1000 ppm concentration, significantly highest per cent growth inhibition over control was recorded in propineb (53.80%) followed by mancozeb (50.63%), chlorothalonil (44.16%), thiram (35.68%), zineb (34.79%) and sulphur (18.78%). At 2000 ppm concentration, mancozeb recorded significantly highest per cent growth inhibition i.e. 69.58% followed by propineb (68.36%), chlorothalonil (59.46%), thiram (35.68%) and zineb (34.79%), copper oxychloride (36.26%) and sulphur (32.51%). At 2500 ppm concentration, mancozeb, propineb and chlorothalonil recorded (92.47%) inhibition of the pathogen. Next best in order of merit was zineb (69.58%) followed by thiram (53.81%) and copper oxychloride (49.13%). Sulphur (40.88%) was comparatively less effective against *P. vexans.* There was no mycelial growth of the pathogen in mancozeb and propineb at 2500 ppm and also significantly lesser growth at 2000 and 1000 ppm. Thus, mancozeb and propineb fungicides proved the most effective against *P. vexans*. Next best fungicide in order of merit was chlorothalonil followed by zineb.

The present findings are confirmed with the results of Mohanty *et al*. (1994) [8] recorded significant growth inhibition of *P. vexans* in mancozeb at 0.1, 0.2 and 0.3 per cent concentrations. Akhtar (2007) [1] revealed that affectivity of mancozeb at 50 ppm was cent per cent inhibition against *P. vexans* isolates Pv 36 and Pv 25, while complete inhibition of isolate Pv 10 was recorded at 500 ppm. Patel (2007) [10] also founded that mancozeb was most effective with mean inhibition of 91.63 per cent at 1000 ppm against *P. vexans*.

Effect of different ready mix fungicides on growth inhibition of *P. vexans*

Results (Plate 3 and Table 3) revealed that all ready mix fungicides tested significantly inhibited mycelial growth of *P. vexans*, over untreated control. Further, per cent mycelial inhibition was increased with increase in concentrations of the fungicides tested. At 500 ppm concentration, highest per cent growth inhibition over control was recorded in pyraclostrobin 12.5% + epoxiconazole 4.7% (73.68%) followed by carboxin 37.5% + thiram 37.5% (72.24%). The remaining fungicides *viz.*, carbendazim 12% + mancozeb 63% (58.65) and pyraclostrobin 5% + mitiram 55% (51.50%) were moderately effective. While captan 70 % + hexaconazole 5 % (32.93%) and hexaconazole 4% + zineb 68% (19.19%), recorded least effective in growth inhibition as compared to other fungicides against *P. vexans*. At 1500 ppm concentration, pyraclostrobin 12.5% + epoxiconazole 4.7% and carboxin 37.5% + thiram 37.5% recorded significantly highest per cent growth inhibition (75.08%) followed by carbendazim 12% + mancozeb 63% (69.05%). pyraclostrobin 5% + mitiram 55% (59.55%) and Captan 70% + Hexaconazole 5% (47.06) were also considerably effective fungicides. hexaconazole 4% + zineb 68% (27.02%) proved comparatively poor in their efficacy against *P. vexans*. At 2000 ppm concentration, carboxin 37.5% + thiram 37.5%, carbendazim 12% + mancozeb 63% and pyraclostrobin 12.5% + epoxiconazole 4.7% were recorded 92.49 per cent growth inhibition of the pathogen. Next best in order of merit was pyraclostrobin 5% + mitiram 55% (72.96%) followed by captan 70 % + hexaconazole 5 % (53.50%). Hexaconazole 4% + zineb 68% (38.80%) was comparatively less effective against *P. vexans*. Different fungicides greatly varied in their efficacy to inhibit the growth of fungus under study. The growth inhibition per cent positively correlated with increase in concentration for all the fungicides tested. It is inferred from results that there was no mycelial growth of the pathogen in carboxin 37.5% + thiram 37.5% and pyraclostrobin 12.5% + epoxiconazole 4.7% at 2000 ppm and also significantly lesser growth at 500 and 1500 ppm compared to the carbendazim 12% + mancozeb 63%. Thus, both the fungicides proved the most effective against *P. vexans*. Next best fungicide in order of merit was carbendazim 12% + mancozeb 63% followed by pyraclostrobin 5% + mitiram 55%. The rest of the fungicides were comparatively less effective against *P. vexans*.

The results are in accords with Patel (2007) [10] investigated that carbendazim 12% + mancozeb 63% was found most effective with mean growth inhibition 88.21 per cent at 1000 ppm against *P. vexans*.

Table 1: Evaluation of systemic fungicides against *P. vexans in vitro*

Sr. No.	Technical name of fungicides	Concentrations (ppm)	Average colony diameter (mm) @	Per cent growth inhibition
1	Carbendazim 50 WP	50	2.80* (7.33)**	70.36
		250	2.48 (5.67)	73.71
		500	0.71 (0.00)	92.51
2	Azoxystrobin 23 SC	50	6.70 (44.33)	29.07
		250	5.73 (32.33)	39.30
		500	5.11 (25.67)	45.83
3	Propiconazole 25 EC	50	3.03 (8.67)	67.94
		250	2.41 (5.33)	74.44
		500	0.71 (0.00)	92.51
4	Hexaconazole 5 EC	50	5.85 (33.67)	38.08
		250	4.60 (20.67)	51.27
		500	4.01 (15.67)	57.49
5	Thiophanate methyl 70 WP	50	7.99 (63.33)	15.37
		250	6.44 (41.00)	31.76
		500	4.52(20.00)	52.08
6	Tebuconazole 25 EC	50	7.40 (54.33)	21.56
		250	5.55 (30.33)	41.18
		500	4.10 (16.33)	56.57
7	Absolute control	-	9.44 (88.67)	-
	S.Em.±		0.07	
	C.D. at 5%		0.20	
	C.V. %		2.53	

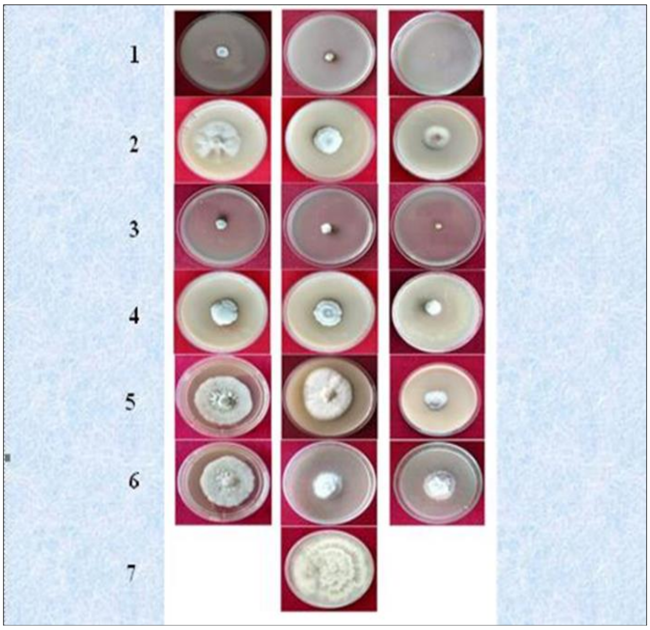
Table 2: Evaluation of non-systemic fungicides against *P. vexans in vitro*

Sr. No.	Technical name of fungicides	Concentrations (ppm)	Average colony diameter (mm) @	Per cent growth inhibition
1	Copper oxychloride 50 WP	1000	7.08* (49.67)**	24.59
		2000	5.99 (35.33)	36.26
		2500	4.78 (22.33)	49.13
2	Mancozeb 75 WP	1000	4.64 (21.00)	50.63
		2000	2.86 (7.67)	69.58
		2500	0.71 (0.00)	92.47
3	Chlorothalonil 75 WP	1000	5.24 (27.00)	44.16
		2000	3.81 (14.00)	59.46
		2500	0.71 (0.00)	92.47
4	Thiram 75 WP	1000	6.04 (36.00)	35.68
		2000	5.18 (26.33)	44.86
		2500	4.34 (18.33)	53.81
5	Zineb 75 WP	1000	6.12 (37.00)	34.79
		2000	4.49 (19.67)	52.20
		2500	2.86 (7.67)	69.58
6	Propineb 70 WP	1000	4.34 (18.33)	53.80
		2000	2.97 (8.33)	68.36
		2500	0.71 (0.00)	92.47
7	Sulphur 80 WDG	1000	7.63 (57.67)	18.78
		2000	6.34 (39.67)	32.51
		2500	5.55 (30.33)	40.88
8	Absolute control	-	9.39 (87.67)	-
	S.Em.±		0.07	
	C.D. at 5%		0.20	
	C.V. (%)		2.68	

Table 3: Evaluation of ready mix fungicides against *P. vexans in vitro*

Sr. No.	Technical name of fungicides	Concentration (ppm)	Average colony diameter (mm) @	Per cent growth inhibition over control
1	Carboxin 37.5% + Thiram 37.5%	500	2.61* (6.33)**	72.24
		1500	2.35 (5.00)	75.08
		2000	0.71 (0.00)	92.49
2	Captan 70 % + Hexaconazole 5 %	500	6.31 (39.33)	32.93
		1500	4.98 (24.33)	47.06
		2000	4.38 (18.67)	53.50
3	Hexaconazole 4% + Zineb 68%	500	7.60 (57.33)	19.19
		1500	6.87 (46.67)	27.02
		2000	5.76 (32.67)	38.80
4	Carbendazim 12% + Mancozeb 63%	500	3.89 (14.67)	58.65
		1500	2.91 (8.00)	69.05
		2000	0.71 (0.00)	92.49
5	Pyraclostrobin 12.5% + Epoxiconazole 4.7%	500	2.48 (5.67)	73.68
		1500	2.35 (5.00)	75.08
		2000	0.71 (0.00)	92.49
6	Pyraclostrobin 5% + Metiram 55%	500	4.56 (20.33)	51.50
		1500	3.81 (14.00)	59.55
		2000	2.54 (6.00)	72.96
7	Absolute control	-	9.41 (88.00)	-
	S.Em.±		0.07	
	C.D. at 5%		0.20	
	CV %		3.03	

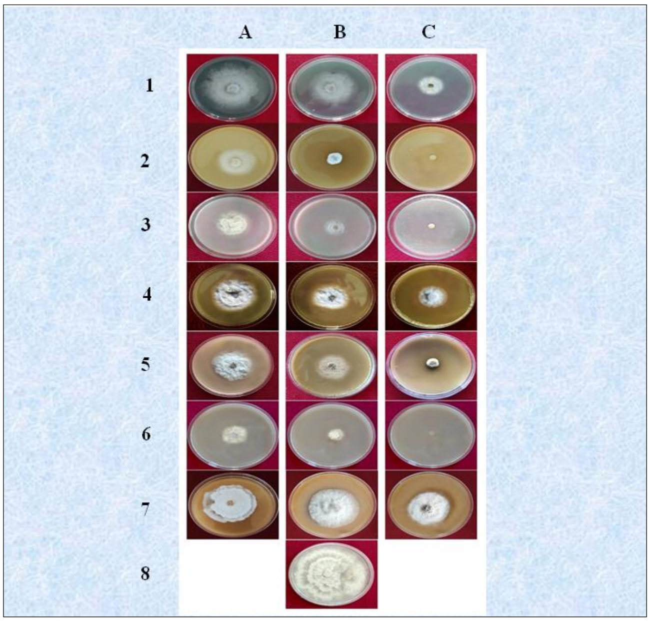
@ Mean of three repetitions
* Figures outside parenthesis are √x+0.5 transformed value
****** Figures in parenthesis are original values



Fungicides
1. Carbendazim 50 WP
2. Azoxystrobin 23 SC
3. Prop is on az ole 25 EC
4. Hexacon azole 5 EC
5. Thiophanate methyl 70 WP
6. Tebuconazole 25 EC
7. Control

Concentration
A. 50 ppm
B. 250 ppm
C. 500 ppm

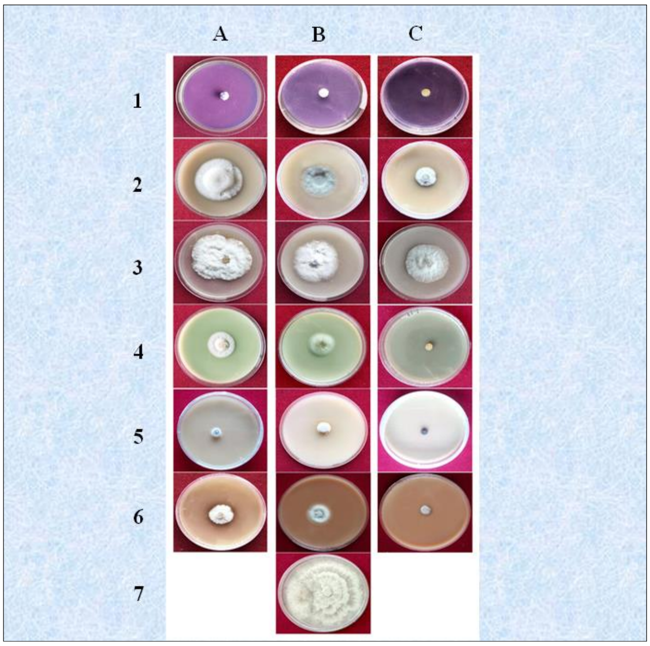
Plate 1: Growth inhibition of *P. vexans* at different] concentrations of systemic fungicides



Fungicides
1. Copper oxychloride 50 WP
2. Mancozeb 75 WP
3. Chlorothalonil 75 WP
4. Thiram 75 VIP
5. Zineb 75 WP 6.Antra col 70 WP
6. Sulphur 80 WP
7. Control

Concentration
A. 1000 ppm
B. 2000 ppm
C. 2500 ppm

Plate 2: Growth inhibition of *P. vexans* at different concentrations of non systemic fungicides



Fungicides Concentration

1. Carboxin 37.5% + Thiram 37.5% WP — A. 500 ppm
2. Captain 70 % + Hexaconazole 5 % WP — B. 1500 ppm
3. Hexaconazole 4% + Zineb 68% — C. 2000 ppm
4. Carbendazim 12% + Mancozeb 63% WP
5. Pyraclostrobin 12.5% + Epoxiconazole 4.7% SE
6. Pyraclostrobin 5% + Alitiram 55%
7. Control

Plate 3: Growth inhibition of *R vexans* at different concentrations of ready mix fungicides

Conclusion
Results concluded that the minimum mycelial growth was recorded in carbendazim and propiconazole were found cent per cent growth inhibition at 500 ppm and also significantly lesser growth at 50 and 250 ppm compared to rest of concentrations. In case of non-systemic fungicides, mancozeb and propineb proved cent per cent growth inhibition of *P. vexans* at 2500 ppm and also significantly lesser growth at 1000 and 2000 ppm. Ready mix fungicides at various concentrations were screened *in vitro* against *P. vexans*, in carboxin 37.5% + thiram 37.5% and pyraclostrobin 12.5% + epoxiconazole 4.7% were found cent per cent growth inhibition at 2000 ppm and also significantly lesser growth at 500 and 1500 ppm compare with other fungicides.

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