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Eco-friendly management of fusarium wilt of bottle gourd

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Abstract
In vitro efficacy of various isolates of bio-agents against wilt of bottle gourd revealed that the isolate *Trichoderma viride* (Sardarkrushinagar) was the most effective against *Fusarium oxysporum* and it was followed by *T. harzianum* (Junagadh), *viride* (Junagadh), *T. viride* (Navsari), *Bacillus subtilis* (Sardarkrushinagar) and *Pseudomonas fluorescens* (Sardarkrushinagar). Among ten phyto - extracts evaluated, the maximum growth inhibition of *F. oxysporum* was recorded with neem extract (52.38%) and it was followed by nilgiri extract (41.62%). Among four oil cakes and three organic manures evaluated, the maximum inhibition of *F. oxysporum* was observed with neem cake (98.75%) and it was followed by poultry manure, castor cake, farm yard manure, cotton cake and vermicompost under *In vitro* condition.

Keywords: Wilt, bio-agent, phyto-extract, organic manures

Introduction
Cucurbitaceae is a plant family commonly known as "gourds" or "cucurbits" having tremendous commercial importance. This group includes cucumber, muskmelon, watermelon, bottle gourd, bitter gourd, sponge gourd, ridge gourd, snake gourd, pointed gourd, round gourd, ash gourd, pumpkin, summer squash, winter squash and a number of other crops mostly of trailing habit.

Bottle gourd (*Lagenaria siceraria* Mol. Standl.) is one of the major vegetable crops belonging to the family *cucurbitaceae.* It is known by many names *viz*., birdhouse gourd, trumpet gourd, calabash gourd, white flowered gourd and dudhi in India. It is a climbing or trailing herb with bottle, oval or dumb-bell shaped fruits. Among fungal diseases, wilt of bottle gourd caused by *Fusarium oxysporum* f. sp. *lagenariae*, is an economically important disease especially under protected cultivation as is responsible for heavy fruit yield losses. The disease also affects other crops of the Cucurbitaceae family *viz.*, melon, squash, cucumber and pumpkin.

The symptoms of wilt are dependent on several factors, including the amount of inoculum in the soil, environmental conditions, nutrients and susceptibility of the host. Wilting is followed by a yellowing of the leaves and finally necrosis. The wilting generally starts with the older leaves and progresses to the younger foliage. Under conditions of sufficiently high inoculum density or a highly susceptible host, the entire plant may wilt and die within a short time. Therefore, with a view to generate scientific information related to this pathogen, the present investigation was proposed.

Material and Methods
In vitro evaluation of different bio-agent against Fusarium wilt pathogen
Different bio-agents were screened for their effectiveness against wilt pathogen by dual culture technique (Table 1). The test bio-agents and pathogen were grown separately on PDA. Sterilized PDA poured aseptically in 90 mm diameter sterilized Petri-plates. Mycelial disc from seven days old actively growing culture of bio-agents and the test pathogen were cut aseptically from the periphery of the colony with the help of sterilized cork borer and placed on solidified PDA approximately 60 mm away from each other. Test pathogen and bio-agent were subjected alone for growth and comparison. All inoculated Petri-plates were incubated at 27 ± 2 °C temperature in an incubator. The observations on radial growth in each Petri-plates were measured periodically and final observation was recorded when the control plate was fully covered with the growth of test pathogen.

The per cent growth inhibition (PGI) was calculated by the following equation:

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

Where,
PGI = Per cent growth inhibition
C = Colony diameter in control (mm)
T = Colony diameter in treatment (mm)

Table 1: List of bio-agents tested against wilt pathogen of bottle gourd *in vitro*

Sr. No.	Name of bio-agent
1.	*Trichoderma viride* (Sardarkrushinagar)
2.	*Trichoderma harzianum* (Junagadh)
3.	*Trichoderma viride* (Junagadh)
4.	*Trichoderma viride* (Navsari)
5.	*Bacillus subtilis* (Sardarkrushinagar)
6.	*Pseudomonas fluorescences* (Sardarkrushinagar)

Bio-efficacy of different phyto-extracts against Fusarium wilt pathogen *in vitro*

The extracts of different plants species *viz.*, bulbs of garlic and onion, leaves of datura, tulsi, heena, castor, custard apple, sarpgandha, nilgiri and neem were evaluated against bottle gourd wilt pathogen.

Healthy fresh leaves, bulbs collected and first washed with sterilized distilled water, then sterilized with 90 per cent methanol and air-dried. Weighted plant material was crushed in the electrically operated mixer and grinder by adding 100 ml distilled water to obtain 1:1 extracts. The material was homogenized for five minutes and filtered through double layer muslin cloth. Then the filter was centrifuged at 5000 rpm for 15 minutes. The supernatant was collected. All the plant extracts were used at 5, 10 and 20 per cent concentration. The plant extract of 5 ml was added to 95 ml of the sterilized warm Potato Dextrose Agar medium for 5 per cent concentration, 10 ml of the plant extract was added to 90 ml of the sterilized warm Potato Dextrose Agar medium for 10 per cent concentration and 20 ml of the plant extract was added to 80 ml of the sterilized warm Potato Dextrose Agar medium for 20 per cent concentration. Flasks containing PDA without extracts served as control.

After solidification of the medium, Petri-plates were inoculated with fungal mycelial discs of seven days old culture of bottle gourd wilt pathogen, raised on PDA. The inoculated Petri-plates were incubated at 27 ± 2 °C temperature for seven days and colony diameter was measured.

The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the equation adopted by Bliss (1934) [2].

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

Where,
PGI = Per cent growth inhibition
C = Colony diameter in control (mm)
T = Colony diameter in treatment (mm)

***In vitro* evaluation of different organic amendments against Fusarium wilt pathogen**

To study the effect of different organic amendments on the growth of wilt pathogen in bottle gourd, the poison food technique was used. A total of seven amendments *viz.*, mustard cake, neem cake, castor cake, cotton cake, farm yard manure (FYM), vermicompost and poultry manure were evaluated at 5, 10 and 20 per cent concentrations. All the amendments were crushed to make a fine powder. Fifty grams powder of each amendment was taken into 250 ml flask and 150 ml water added to the flask. All these flasks were plugged with cotton and allowed for decomposing the material for 15 days. After 15 days, the material was strained with a muslin cloth to obtain the extract. The strained liquid was autoclaved at 1.045 kg/cm2 pressure for 20 minutes and considered as cent per cent concentration (standard solution). The measured quantity of a standard solution of the organic amendments were incorporated separately in melted sterilized PDA medium in conical flasks aseptically at the time of pouring the medium to obtain desired concentrations. The medium was shaken well to give uniform dispersal and then poured about 20 ml in each sterilized Petri-plates. After solidification of the medium, the Petri- plates were inoculated in the centre by placing seven days old mycelial discs and then incubated at 27 ± 2 °C temperature. A control was also maintained by growing the pathogen on soil amendment free medium.

The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the equation adopted by Bliss (1934) [2].

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

Where,
PGI = Per cent growth inhibition
C = Colony diameter in control (mm)
T = Colony diameter in treatment (mm)

Results and discussion

Per cent growth inhibition of *F. oxysporum* by bio-agents *In vitro*

The results presented in table 2 revealed that all the bio-agents significantly inhibited the mycelial growth of the *F. oxysporum*. Among the six bio-agents tested, the highest per cent radial growth inhibition of 66.67 per cent was recorded with *T. viride* (Sardarkrushinagar) which was closely followed by *T. harzianum* (Junagadh) (65.56%), *T. viride* (Junagadh) (64.45%), *T. viride* (Navsari) (62.23%) and *B. subtilis* (Sardarkrushinagar) (60.00%). All these bio-agents were at par with each other in inhibiting the mycelial growth of *F. oxysporum.* Although, *P. fluorescens* (Sardarkrushinagar) was comparatively inferior to all the other bio-agents, as it recorded only 57.78 per cent growth inhibition of the test fungi.

Table 2: Per cent growth inhibition of *F. oxysporum* by bio-agents *in vitro*

Sr. No.	Name of the bio-agent	Growth of * pathogen (mm)		Growth inhibition over control (%)
1.	*Trichoderma viride* (Sardarkrushinagar)		30	54.74**a (66.67)*
2	*Trichoderma harzianum* (Junagadh)		31	54.07ab (65.56)
3.	*Trichoderma viride* (Junagadh)		32	53.40ab (64.45)
4.	*Trichoderma viride* (Navsari)		34	52.08bc (62.23)
5.	*Bacillus subtilis* (Sardarkrushinagar)		36	50.77cd (60.00)

6.	*Pseudomonas fluorescens* (Sardarkrushinagar)		38	49.47d (57.78)
	S.Em±			1.49
	C.D. at 5%			2.14
	C.V. %			2.71

*Average of three replications.
**Arc-sin transformed values.
*Figures in parentheses are original values.
Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test.

Present investigation is contradictory of Goswami and Islam (2002) studied the antagonistic effects of *Trichoderma* spp. and *B. subtilis* on *F. oxysporum* f. sp. *lycopersici* causing wilt of tomato *In vitro.* Among all the antagonists evaluated, *T. viride* and *B. subtilis* were found to be effective in suppressing the growth of pathogen followed by *T. harzianum* and *T. kobningii.* Sangle and Bambawale (2004) [11] studied *In vitro* evaluation of the antagonists against wilt of sesame caused by *F. oxysporum* f. sp. *sesame*. *T. viride* reduced the growth of the pathogen by 83.18 per cent whereas, *T. harzianum* reduced it by 79.54 per cent after seven days of inoculation.
Honmane (2007) [6] studied the anthurium wilt caused by *F. moniliforme* and reported that *T. viride* was the most effective in suppressing the growth (87.41%) of *F. moniliforme In vitro* conditions. Verma *et al*., (2018) [12] studied *In vitro* efficacy of antagonists and found that mycelium inhibition of Fusarium wilt of tomato was higher with *T. viride* (52.31%) as compared to *Trichoderma* spp. (47.09%).

Per cent growth inhibition of *F. oxysporum* by phyto-extracts *in vitro*

A total of ten extracts were screened for their antifungal properties at three different concentration (5%, 10%, 20%). The neem extract found the most effective, it gave 75.74 percent growth inhibition at 20 per cent concentration, 44.76 per cent growth inhibition at 10 per cent concentration and 36.63 per cent growth inhibition at 5 per cent concentration. It was followed by nilgiri leaf extract, it gave 65 per cent growth inhibition at 20 per cent, 32.47 per cent growth inhibition at 10 per cent concentration and 27.39 per cent growth inhibition at 5 per cent concentration (Table 3).

Table 3: Per cent growth inhibition of *F. oxysporum* by phyto-extracts *In vitro*

Sr. No.	Phyto-extract	Growth inhibition (%) * Concentration (%) 5	10	20	Mean
1.	Garlic	20.22**mn (11.38)*	30.84ij (26.30)	41.09e (43.21)	30.72d (26.96)
2.	Onion	19.16n (10.81)	23.96l (16.52)	27.60k (21.49)	23.57g (16.27)
3.	Datura	23.52l (15.95)	30.72ij (26.12)	35.25h (33.34)	29.83e (25.14)
4.	Tulsi	18.79n (10.44)	39.15f (39.87)	48.00c (55.23)	35.31c (35.18)
5.	Heena	24.06l (16.67)	27.51k (21.36)	32.45i (28.80)	28.01f (22.28)
6.	Castor	19.44mn (11.12)	22.09lm (14.20)	45.53d (50.93)	29.02de (25.42)
7.	Custard apple	18.78n (10.43)	21.96lm (14.02)	23.53l (15.99)	21.42h (13.48)
8.	Sarpgandha	22.96l (15.25)	29.95j (24.94)	38.47fg (38.72)	30.46de (26.30)
9.	Nilgiri	31.54ij (27.39)	34.72h (32.47)	53.73b (65.00)	40.00b (41.62)
10.	Neem	37.23g (36.63)	41.98e (44.76)	60.50a (75.74)	46.57a (52.38)
Mean		21.45^{c} (16.61)	27.56^{b} (26.06)	36.95^{a} (42.84)	-
		Phyto-extract	Concentration	Phyto-extract × Concentration	
S. Em. ±		0.39	0.21	0.67	
C.D. at 5%		1.17	0.61	2.01	
C.V. %		4.35			

*Average of three replications.
**Arc-sin transformed values.
*Figures in parentheses are original values.
Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test.

Present investigation is contradictory of Jan *et al.*, (2015) [7] evaluated five plant extracts *viz*., *Lantana camara*, *Eucalyptus globulus*, *Ocimum sanctum*, *Calotropis gigantea* and *Azadirachta indica* against *F. oxysporum* f. sp. *gladioli*, the causal pathogen of gladiolus wilt *In vitro*. Among evaluated plant extracts, the maximum mycelial growth inhibition was recorded with *Azadirachta indica* (43.90%) and it was followed by *Ocimum sanctum* (40.79%).
The phyto-extracts / botanicals *viz. Allium cepa*, *Lantana camara*, *Ocimum sanctum*, *Gliricidia sepium*, *Azadirachta indica*, *Allium sativum*, *Bougainvillea spectabilis*, *Moringa oleifera*, *Eucalyptus globulus*, *Pongamia pinnata*, *Vinca rosea* and *Asparagus racemosus* were evaluated *In vitro* for their antifungal activities against wilt of pigeon pea, (*F. udum)* using poison food technique. The highest mycelial growth inhibition was recorded with *Azadirachta indica* (77.23%) and it was followed by *Allium sativum* (76.11%) (Ghante *et. al*., 2019) [4].
A total of six different plants extracts of neem, tulsi, aak, eucalyptus, onion and garlic were evaluated at different concentrations by poisoned food technique against Fusarium wilt pathogen of brinjal to test their efficacy. The neem leaf extract was found to be the most effective in inhibiting the mycelial growth of *F. oxysporum* f. sp. *melongenae* at 50 per cent concentration (Jatav *et. al*., 2019) [9].

Per cent growth inhibition of *F. oxysporum* by organic amendments *in vitro*

Among the seven organic amendments, the neem cake found the most effective, it recorded 99.50 per cent growth

inhibition at 20 per cent and 10 per cent concentration and 97.24 per cent growth inhibition at 5 per cent concentration. It was followed by castor cake and obtained 91.79 per cent growth inhibition at 20 per cent concentration, 80.74 per cent growth inhibition at 10 per cent concentration and 79.12 per cent growth inhibition at 5 per cent concentration.

Table 4: Per cent growth inhibition of *F. oxysporum* by organic amendments *In vitro*

Sr. No.	Organic amendment	Per cent growth inhibition*			Mean
		Concentration (%)			
		5	10	20	
1	Mustard cake	16.88**j (8.49)*	21.47i (13.44)	27.97h (22.02)	22.10f (14.65)
2	Neem cake	80.47a (97.24)	85.94a (99.50)	85.94a (99.50)	84.12a (98.75)
3	Castor cake	62.83d (79.12)	63.98d (80.74)	73.37b (91.79)	66.73b (83.88)
4	Cotton cake	54.15f (65.70)	54.57f (66.38)	67.67c (85.54)	58.80d (72.54)
5	Farm yard manure	54.60f (66.45)	57.69e (71.42)	64.35d (81.25)	58.88d (73.04)
6	Vermicompost	44.67g (49.44)	45.34g (50.59)	53.32f (64.32)	47.78e (54.78)
7	Poultry manure	54.47f (66.22)	64.87d (81.95)	67.91c (85.77)	62.42c (77.98)
Mean		46.04^{c} (61.81)	49.27^{b} (66.29)	55.10^{a} (75.74)	-
		Organic amendment	Concentration		Organic amendment × concentration
S. Em. ±		0.42	0.26		0.72
C.D. at 5%		1.25	0.76		2.16
C.V. %		2.62			

*Average of three replications.
**Arc-sin transformed values.
*Figures in parentheses are original values.
Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test.

Present investigation is contradictory of the maximum inhibition (60.49%) of *F. oxysporum* f. sp. *ciceri* was recorded at concentration of 7 per cent of neem cake, followed by 5 per cent (31.94%) and 3 per cent (8.89%) under *In vitro* condition (Animisha *et al.,* 2012) [1]. Patel and Patel (2012) [10] tested eight organic amendments against *F. udum In vitro* and reported significantly the highest inhibition in the growth of *F. udum* with sesame cake (46.74%) followed by neem cake (42.50%) at 30 per cent concentration.

Jat and Ahir (2017) [8] evaluated different organic amendments *viz.,* neem cake, vermicompost, goat and sheep manure, mustard cake and wool waste at different concentrations *i.e.,* 10, 20 and 30 per cent against *F. solani* causing Indian Aloe root rot under laboratory condition by poisoned food technique**.** Among them, the neem cake extract was the most effective with 79.00 per cent growth inhibition, followed by vermicompost (74.80%) and goat and sheep manure (68.3%). Dhivya *et al.,* (2017) [3] found that neem cake extract (10%) recorded the maximum reduction of mycelial growth (80.77%) over control, followed by mahua cake extract (76.33%). The minimum reduction (62.11%) was recorded with coconut cake.

Conclusion

Among different isolates of bio-agents evaluated, the local isolate *Trichoderma viride* (Sardarkrushinagar) was proved to be the highly efficacious against *F. oxysporum* followed by *T. harzianum* (Junagadh), *T. viride* (Junagadh), *T. viride* (Navsari), *B. subtilis* (Sardarkrushinagar) and *P. fluorescens* (Sardarkrushinagar).

Among ten phyto-extract evaluated, the neem extract proved its superiority, which inhibit fungal growth by 36.63 per cent at 5 per cent, 44.76 per cent at 10 per cent and 75.74 per cent at 20 per cent concentrations. In nilgiri extract the per cent inhibition was recorded as 27.39 per cent at 5 per cent, 32.47 per cent at 10 per cent and 65.00 per cent at 20 per cent concentrations with nilgiri leaf extract. Among seven organic amendments, the highest inhibitory effect was recorded with neem cake at 10 and 20 per cent concentrations with mean growth inhibition of 99.50 per cent. The next better amendments in order of merit were castor cake, poultry manure, cotton cake, farm yard manure and vermicompost at 20 per cent concentration with growth inhibition of 91.79, 85.77, 85.54, 81.25 and 64.32 per cent, respectively. These findings are highly important to develop the eco-friendly management strategies for wilt disease of bottle gourd at field level.

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