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Assessment of fungicides, bio-agent and botanicals against *Fusarium* wilt of betelvine

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Abstract
Wilt of betelvine is an important root disease of betelvine inflicting heavy losses in crop. The present investigation was carried out to test the efficacy of fungicides, botanicals and bio-agents *in vitro*. Fungitoxic activities of tested fungicides were proved against *Fusarium solani.* Wide differences were existed among the fungicides for its efficacy. On 7th DAI, absolutely no growth was noted in carbendazim and carboxin+thiram while mancozeb, propineb and copper oxychloride noted 32.22, 30.29 and 39.51 mm radial growth with inhibition of 53.51, 56.30 and 43.00% as against 69.32 mm mycelia growth in control. Bio-efficacy of bio-agent tested by dual culture technique and results revealed that maximum mycelial growth inhibition of *F. solani* was recorded in *T. harzianum* 59.15. However, *T. asperellum* recorded 52.41 followed by *T. atroviride* 38.72, *P. fluorescens* 26.52 and *B. subtilis* 17.50%. In respect of plant extract, minimum growth and higher inhibition was noted ginger (26.87%) followed by garlic (25.53%) and turmeric (14.17%) and lowest inhibition occurred in onion (7.18%) and neem (6.38%).

Keywords: Betelvine, *Fusarium solani*, fungicides, bio-agent, *Trichoderma* spp.

Introduction
Betelvine or 'Pan' (*Piper betel* L). is a perennial dioecious, shade loving root climber native of Malaysia is grown in India since ancient times. It is cultivated under tropical conditions having a cool, shade, humid climate grown throughout the country and important horticultural crop of aesthetic and commercial values.

It is mostly use to chew with sliced areca nut, slaked lime, coriander, clove, cardamom, sweetener, coconut scrapings etc. It is cultivated in hotter and damper part in country following the traditional methods in India on about 55,000 hectare with an annual production worth about Rs. 9000 million. Focusing on traditional and medicinal use of *Piper betel* cures many diseases and reduces the oral cancer which actually happens due to sliced arecanut, slaked lime not because of betel leaves. Leaves are rich in many nutrients like water, energy, protein, fats, fiber, calcium and iron etc. and the antioxidants present are flavonoids, tannins, saponins and alkaloids. It helps in curing various diseases like diabetes, obesity, wound healing, itches, mastitis, mastoiditism, leucorrhoea, ringworm, hypertension, voice problem, constipation, headache, hysteria, conjunctivitis, swelling of gums, rheumatism, cut and injuries etc (Richa and Neetu Singh, 2017) [7].

In Maharashtra, the cultivation of betelvine is restricted to an area of about 2,300 hectares including Sangli, Satara, Kolhapur, Thane, Pune, Nashik, Jalgaon, Buldhana, Dhule, Aurangabad, Akola, Amravati and Nagpur districts.

Betelvine cultivation has major disease problem in Vidarbha, which are quite severe in rainy season including root and foot rot, wilt, die back, leaf spot and blight due to various fungi and root knot nematodes but root disease among them is serious one. Chawdhury (1944) reported 2-47 per cent losses due to *Fusarium solani* are predominant in Maharashtra as an serious hazards to betel crop. In present study various fungicides, plant extracts and bio-agents were assayed for efficient management strategies of root rot pathogens.

Materials and Methods
Isolation
For the isolation of pathogen Potato dextrose agar media was used. Approximately 20 ml autoclaved PDA was poured in each sterilized Petri dish and allowed to solidify. The diseased plant specimens were cleaned properly. The diseased portion was cut into small bits along with

the healthy portion with sterilized blade and transferred into sterilized Petri plates containing 0.01% sodium hypochlorite solution for surface sterilization. After a minute the bits were transferred to sterilized water and washed by three sequential changes in sterilized distilled water to remove the traces of sodium hypochloride. Bits were blot dried by keeping them on sterilized filter paper to absorb the excess water. Three bits were aseptically transferred to solidified PDA medium in sterilized Petri dish at equidistance and incubated at room temperature (27 ^{0}C $\pm$ 1 ^{0}C). All the operations were carried out aseptically and growth of organism was observed regularly. The fungus growth observed around the infected bits was transferred on plates. The slides were prepared and examined under research microscope for identification.

***In -vitro* evaluation of fungicides**

Poisoned food technique was used to evaluate the efficiency of fungicides against pathogens. Potato dextrose agar medium was prepared and distributed at the rate of 100 ml in 250 ml conical flask, autoclaved 1.05 kg/cm^2 for 15 min. Then before solidification of media different fungicides with desired concentration were incorporated aseptically in different flasks. These flasks shaken thoroughly and poured in Petri plates 20 ml/plate likewise three plates for each treatment were poured. One set of three plates was poured without any fungicides to serve as a control. After solidification of medium, the plates inoculated with eight days old pathogens separately. Six mm diameter mycelial disc selected from peripheral growth of the plate by sterilized cork borer were used for inoculating the plates by keeping one disc per plate in the centre in inverted position, so as to make the mycelia growth touch the surface of medium. The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogens on medium was recorded and percent inhibition in each treatment was calculated (Vincent, 1927) [9] by using following formula.

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

Where,
I = Per cent inhibition of mycelial growth
C = Growth of mycelium in control (mm)
T = Growth of mycelium in treatment (mm)

***In -vitro* evaluation of bio-agents**

The lawn culture of test fungi and bio-agents *i.e. Trichoderma harzianum, T. asperellum and T. atroviride* were prepared. Autoclaved PDA medium was poured in the sterilized Petri plates and allowed to solidify for obtaining leveled surface. The plates were inoculated with the culture of test fungi and bio-agents after solidification of media and then plates were incubated at room temperature for seven days.

Bacterial bio-agent *Pseudomonas fluorescens* was prepared by inoculating a loopful culture in sterilized conical flask containing hundred ml of nutrient broth. Broth culture was incubated at room temperature for three days. Five mm disc of one week old test fungus and bio-agent lawn culture was cut with the help of cork borer lifted and transferred in Petri plates, containing autoclaved solidified medium. In each Petri plates, four discs of bio-agents were inculated at four peripheral points of the plates and the test fungi was placed in centre of Petri plates. In case of *Pseudomonas fluorescens*, a three days old culture was streaked around the disc of test fungus. The test fungi grown in same condition on medium without bio-agents served as control. The treatments were replicated thrice. All these plates were incubated at room temperature for three, five and seven days. After an expiry of seven days incubation period the radial mycelial growth of test fungi was measured in treated and controlled plates on 3rd, 5th and 7th days and inhibition per cent was calculated Vincent (1927) [9].

Preparation of aqueous plant extracts

The plant leaves extract were prepared by adopting aqueous extracting method. The standard aqueous leaf and clove corms extract of the selected botanicals were obtained by grinding the washed plant leaves (100 g) in mortar and pestle in presence of equal amount of sterilized distilled water (100 ml). Prepared leaves extract were filtered through three folds of muslin cloth, make up the volume and treated as 100% extract. Colony diameter was recorded in mm and per cent of mycelial inhibition was calculated based on the average of colony diameter. The data of mycelial growth was also subjected to statistical analysis and conclusions were drawn.

Results and Discussion

***In -vitro* evaluation of fungicides**

Efficacy of 5 fungicides at respective concentration was tested *in- vitro* by following poison food technique for mycelial growth of *Fusarium solani.*

Table 1: Efficacy of fungicides against *Fusarium solani* by poisoned food techn

Treatment No.	Fungicides	Concentration (%)	Mycelial growth (mm)			Growth inhibition (%)
			3	5	7	
T1	Mancozeb	0.2	19.00	25.85	32.22*	53.51
T2	Carbendazim	0.1	00.00	00.00	00.00	100.00
T3	Carboxin+Thiram	0.1	00.00	00.00	00.00	100.00
T4	Propineb	0.2	22.00	24.86	30.29	56.30
T5	Copper oxychloride	0.3	25.50	32.68	39.51	43.00
T6	Control		27.50	40.45	69.32	
	'F' test				Sig.	
	SE(M)±				0.36	
	CD (p=0.01)				1.55	

*Mean of four replications *DAI- Day after inoculation.

A Fungitoxic activity of test fungicides was proved against *Fusarium solani* and the data are presented in Table 1. Wide differences were existed among the fungicides for its efficacy. On 7th DAI, absolutely no growth was noted in carbendazim and carboxin+thiram i.e 100% inhibition while mancozeb, propineb and copper oxychloride noted 32.22, 30.29 and 39.51 mm radial growth with inhibition of 53.51, 56.30 and 43.00% as against 69.32 mm mycelia growth in control.

Similar trend was noted on 3rd and 5th DAI. The present investigation correlates with findings of Ramaswamy (2000)

[6], Nasreen Sultana and Ghaffer (2010) [5] and Kendre and Ingle (2016) [4]. They have observed that combi product gave significant reduction in growth of *Fusarium spp.* Chemical constituents present in the test fungicides are having detrimental effects they have reduced the growth and resulted in maximum inhibition.

In -vitro* evaluation of bio-agents against *Fusarium solani

Efficacy of bio-agent against *F. solani* was tested by *dual* culture technique and results obtained are presented in Table 2. Among five bio-agents, maximum mycelial growth inhibition 59.15% was recorded in *Trichoderma harzianum.* However, *T. asperellum* recorded 52.41 followed by *T. atroviride* 38.72, *P. fluorescens* 26.52 and *B. subtilis* 17.50%. These results are in accordance with the findings of Kendre and Ingle (2016) [4] and Shankaranavar and Somashekhara (2017), who recorded that *Trichoderma harzianum* inhibited maximum growth of *Fusarium solani* incitant of betelvine wilt.

Table 2: Efficacy of bio-agents against *Fusarium solani* by dual culture technique (DAI)

Treatment no.	Bio-agents	Mycelial growth (mm)			Growth inhibition (%)
		3	5	7	
T1	*Trichoderma harzianum*	21.50	28.45	32.88	59.15
T2	*Trichoderma asperellum*	28.50	32.48	38.30	52.41
T3	*Trichoderma atroviride*	24.50	36.67	49.32	38.72
T4	*Pseudomonas fluorescens*	29.50	39.69	59.14	26.52
T5	*Bacillus subtilis*	29.89	45.49	66.40	17.50
T6	Control	34.50	54.50	80.48	
	'F' test			Sig.	
	SE(M)±			0.38	
	CD (p=0.01)			1.62	

*Mean of four replications

The restriction of fungal growth by these tested antagonists is due to mycoparasitism, antibiosis and lysis of the pathogen causing disease in betelvine. Antagonist secretes various toxic enzymes might degraded the hyphal growth and disintegration of mycelium of pathogen. In some cases competition among pathogen and antagonist occurs and due to the starvation their will be reduction in multiplication of pathogen resulted in management and likely to restrict the disease complex in betelvine.

Efficacy of plant extracts

Five plant extracts were evaluated against *Fusarium Solani* by using poisoned food techniques.

Table 3: Efficacy of plant extracts against *Fusarium Solani* by poisoned food technique (DAI).

Treatment no.	Plant extracts**	Mycelial growth(mm)			Growth inhibition (%)
		3	5	7	
T1	Neem (*Azadirachta indica*)	24.50	45.96	55.05	6.38
T2	Turmeric (*Curcuma longa)*	13.00	37.87	50.47	14.17
T3	Garlic (*Allium* sativum)	22.50	30.58	45.55	22.53
T4	Ginger *(Zingiber officinale)*	18.00	36.47	43.00	26.87
T5	Onion (*Allium cepa*)	21.50	38.00	54.58	7.18
T6	Control	25.00	48.98	58.80	
	'F' test			Sig.	
	SE(M)±			0.32	
	CD (p=0.01)			1.36	

*Mean of four replications **(All extracts tested @ 10%)

Efficacy of plant extracts against radial mycelial growth of *Fusarium solani* are given in Table 3. Plant extracts have profound and significant effect on radial mycelial growth of the fungus. Highest growth inhibition of *F. solani* due to ginger (26.87%) followed by garlic (25.53%) and turmeric (14.17%) and lowest growth inhibition occurred in onion (7.18%) and neem (6.38%).

The observations noted at 3rd and 7th DAI revealed that the minimum i.e. 13.00 mm and 18.00mm growth was noted on 3rd day in turmeric and ginger while 30.58 and 36.47 mm radial mycelial growth in garlic and ginger respectively increased mycelia growth with duration i.e extended growth might be due to loss in antifungal properties of constituents in extracts.

Arun *et al.* (1995) [1] and Bansal and Gupta (2000) [2] stated that garlic bulb extract was superior in inhibiting the growth of *Fusarium* spp. and thus, the results are on the similar line of findings of earlier worker. The plant extracts constitutes various toxic elements i.e. alkaloids, phenols and harmful enzymes might have restricted the growth of pathogen causing diseases in plant.

Conclusions

The salient features of the present investigation are as under

1. Maximum inhibition of *F. solani* was recorded in Carbendazim and carboxin + thiram.
2. Bio-agents *Trichoderma harzianum, T. asperellum* and *T. atroviride* were found most effective against *F. solani.*
3. Highest growth inhibition of *F. solani* due to ginger (26.87%) followed by garlic (25.53%) and turmeric (14.17%) and lowest inhibition occurred in onion (7.18%) and neem (6.38%).

References

1. Arun AC, *et.al.* Effects of alicin of garlic extracts begonia on two fungi. Indian J Myco. Plant. Path. 1995, 259.

2. Bansal RK, Gupta R. Evaluation of plant extracts against *Fusarium oxysporum*, wilt pathogen of fenugreek. Indian Phytopath. 2000; 55(1):107-108.
3. Chowdhury S. Diseases of pan (*Piper betle*) in Sylhet, Assam. IV. Rhizoctonia root rot. Proc. Indian Aca. Sci. 1944; B20:220-234.
4. Kendre VK, Ingle RW. Diseases of *Piper longum* Linn. and their management thesis, 2016.
5. Nasreen Sultana, Ghaffer. Integrated management of Fusarium wilt of chickpea (*Cicer arietinum* L.) cause by *Fusarium oxyporum* f. sp. ciceri with microbial antagonist, botanical extracts and fungicide. African journal of biotechnology. 2010, 2013; 12(29):4699-4706.
6. Ramaswamy GR. Fungicide resistance and sensitive strain of *F. solani* f. sp. piperis, the causal agent of root rot of betelvine. Plant disease research. 2000; 15(2):168-176.
7. Richa, Neetu Singh. Betel leaf product preparation and its nutritional analysis. Asian Journal of Science and Technology. 2017; 08(11):6487-6489.
8. Shanakaranawar, Somashekra. Studies on etiology and management of Betelvine (*Piper betel.* L) wilt (*Fusarium solani*), 2017.
9. Vincent JM. Distoration of fungal hyphae in presence of certain inhibitors. Nature. 1927; 15:850.