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Research Article

Management of Fusarium wilt of tomato (Pusa Ruby) by plant extracts and fungicides

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Abstract
Fusarium wilt disease of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the most important and widespread disease of the cultivated tomato.The yield loss in tomato in temperate region of India due to this disease is 10 to 90 percent. Investigation was undertaken to screen out the fungicides *viz*., Azoxystrobin(T_1), Propineb(T_2), Thiophanate Methyl (T_3), Difenoconazole(T_4), Mancozeb(T_5), Mancozeb + Thiophanate Methyl(T_6), Boscolid+Pyraclostrobin(T_7) and control (T_8) and plant extracts *viz*, onion bulb(T_9), ,ginger rhizome(T_{10}), garlic clove(T_{11}), neem leaf (T_{12}), ashwagandha leaf(T_{13}), sarpgandha leaf(T_{14}), ashok leaf(T_{15}) and control (T_{16}) , against wilt of tomato under *in vivo* and *in vivo* conditions. Ginger rhizome extract (T_{10}) was found very promising as it produced 82.2 % growth inhibition of *F. oxysporum*f.sp. *lycopersici*at @t 15 % concentration followed by garlic clove(T_{11}) (76.1%). Two soil drenching withgarlic clove(T_{11}), @ 15 percent showed minimum disease incidence of (16.6%) with maximum yield (435.3 q/ha) followed by ginger rhizome(T_{10}) extract (18.4% and 428.2 q/ha). Mancozeb + Thiophanate Methyl(T_6), Difenoconazole(T_4) and Thiophanate Methyl(T_6) completely inhibited the radial growth and sporulation of *Fusarium oxysporum*f.sp. *lycopersici* after 144 hrs of incubation. Two soil drenching of Mancozeb +Thiophanate Methyl(T_6), @ 15 per cent was also found the best for managing the disease as minimum disease incidence (5.3%) and highest yield (470.9 q/ha) was recorded.

Keywords: Fungicide, *Fusarium oxysporum*f.sp.*lycoperisci,* Plant extract, Tomato, Wilt

INTRODUCTION

Fusarium wilt disease of tomato caused by *Fusarium oxysporum*f. sp.*lycopersici* is one of the most important and widespread disease of the cultivated tomato .Vascular wilting in tomatoes alone causes yield losses of 30-40%, and losses can reach up to 80% under adverse weather conditions in India (Nirmaladevi *et al*, 2016; Sidharthan *et al*, 2018). Management of this pathogen is difficult due to endogenous growth and long-term persistence in soil (Borrero *et al.,* 2006; Himabindu and Kumar 2021). The application of plant extracts and fungicides is the method of choice for farmers to manage yield loss. Previously, workers judged the potential of such plant extracts and fungicides (Sultana and Ghaffar, 2013; Fareed *et al*., 2015, Poussio, 2018, Khatun *et al.* 2020).The use of synthetic fungicides is effective but exerts a negative impact on the soil health and environment. The demand for plant-based therapeutics is increasing in India as they are natural products that are easily available and have no harmful effects (Poussio, 2018). This study aimed to examine the management of *Fusarium* wilt of tomato by plant extracts and fungicides and their effect on tomato yield *in vivo.*

MATERIALS AND METHODS

Isolation and identification

Tomato (Pusa Ruby) plants exhibiting typical symp-

toms of Fusarium wilt were collected from the experimental field, Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidyalaya Jabalpur for isolation and identification. The contaminated plant portions were chopped into small pieces and surface sterilized with 0.1 percent Mercuric Chloride solution before being extensively rinsed three to four times with sterilized water to remove Mercuric Chloride residues. The pieces were placed in Petri dishes containing potato dextrose agar medium and incubated for 7 days at 25 0C. Pure colonies were isolated in aseptic conditions from injected petriplates separately.

***In vitro* evaluation of plant extracts**

In order to find out the efficacy of different plant extracts against Fusarium wilt, seven plant extracts *viz*., onion bulb (*Allium cepa*)(T_9); ginger rhizome (*Zingiber officinale*))(T_{10}); garlic clove (*Allium sativum*))(T_{11}); neem leaf (*Azadirachta indica*))(T_{12}); ashwagandha leaf (*Withania sominifera*))(T_{13}); sarpgandha leaf (*Rauvolfia serprentina*) (T_{14}); ashok leaf (*Polyalthia longifolia*))(T_{15}) were used applying poison food techniques. Fresh leaves and bulbs were carefully collected and cleaned in clean water: 100 g of each washed plant material was ground in Pestle and Mortar with an equal quantity (100ml) of sterilized water (1:1V/W) and heated at 80 C for 10 minutes in hot water both. The ingredients were filtered through double-layered muslin cloth, then sterilized Whatman No. 1 filter paper, and the filtrate produced formed the standard plant extract solution (100%). To obtain 5%, 10%, and 15% concentrations of plant extract, 95, 90, and 85 ml of sterilized potato dextrose agar media were added to get 5, 10, and 15% concentrations of plant extract (Sahu *et al* ,2020). Seven plant extracts (T_9 - T_{15}) having three replications were maintained. Five mm discs of 7 days old Laboratory culture of *F. oxysporum*f. sp.*lycopersici* were cut with sterilized cork borer and placed in the centre of plant extract amended petriplates. The control petriplates having PDA alone were inoculated in the same manner. Plates were incubated at 28^0 for 144 hours, and radial growth and sporulation of the test fungus were observed after 15 days. The recorded data on radial growth and sporulation were translated into percent inhibition (Vincent, 1947).

***In vivo* evaluation of plant extracts**

The seven plants's extracts were further evaluated at 15 % concentration as soil drenching on wilt of tomato under field conditions. The experiment was conducted in Plant Pathology research field, Jawaharlal Nehru Krishi Vishwavidyalaya- Jabalpur (M.P.), during 2018-19 in Randomized Block Design with three replications using variety Pusa Ruby with row spacing of 50 x 50 cm and plot size of 2.5 x 3 m^2.The sowing took place on November 27, 2018. Standard plant extracts were produced in cold water using the above-mentioned procedure. After disease start, the extracted extract was diluted to 15% by water and soaked around the root zone in the soil. The soaked plot simply functioned as a check. Two successive drenches were administered at 15-day intervals. Observations on disease incidence were recorded at different intervals using the formula recommended by Masood *et al. (*2010). The yields (q/ha) were recorded after harvest of the crop. Data were analyzed statistically for RBD, CRD and software used was OPSTAT.

***In vitro* evaluation of fungicides**

Seven fungicides, namely Azoxystrobin (T_1) (0.1%), Propineb(T_2) (0.2 %), Thiophanate Methyl(T_3) (0.1%), Difenoconazole(T_4) (0.1%), Mancozeb(T_5) (0.2%), Mancozeb + Thiophanate Methyl(T_6) (0.15%), Boscolid+Pyraclostrobin(T_7) (0.1 %) along with control (T_8) were evaluated against *Fusarium oxysporum*f. sp. *lycopersici*by "Poison Food Techniques" as described by Morton and Straube (1955). Dose was set based on a review of previous studies by Indian and international scientists. Each fungicide was well mixed with 100 ml of sterilized PDA medium placed in 200 ml flakes. It was then well blended before being put into Petriplates and allowed to solidify. Each therapy was repeated three times. The PDA-only control petriplates were infected in the same way. Five mm diameter of pathogen colony from seven days old Laboratory culture of *Fusarium oxysporum*f. sp. *lycopersici was* cut with the help of a cork borer and inoculated at the center in each Petridish. The inoculated Petri-dishes were incubated at 28±1ºC and observation of radial growth of test fungus was recorded after 144 hours.

***In vivo* evaluation of fungicides**

Fungicides were further evaluated as soildrenching under field condition onwilt of tomato under field conditions. Plant Pathology research field, JNKVV Jabalpur (M.P.)during 2018-19 in Randomized Block Design with three replications using variety Pusa Ruby with row spacing of 50 x 50 cm and plot size of 2.5 x 3 m^2**.**Sowing took place on November 27, 2018. Following disease onset, standard fungicide solutions were made and soaked around the crop's root zone. The soaked plot simply functioned as a check. Two successive drenches were administered at 15-day intervals. Observations on illness prevalence were recorded at different intervals using the formula recommended by Masood *et al.* (2010). The yields (q/ha) were recorded after the harvest of the crop. Data were analyzed statistically for RBD, CRD and the software used was OPSTAT.

RESULTS AND DISCUSSION

Evaluation of fungicides

All the fungicides significantly inhibited the radial growth and sporulation of *F. oxysporum*f.sp.*lycopersici in vitro* (Table1,Fig. 1). Thiophanate Methyl (T_3), Difenoconazole(T_4), Mancozeb + Thiophanate Methyl (T_6), were found most effective fungicides which completely inhibited the radial growth and sporulation of *F. oxysporum*f.sp.*lycopersici* after 144 hrs of incubation under *in vitro* condition. Least inhibition was recorded in Azoxystrobin (55.2%). Two soils drenching of Mancozeb +Thiophanate Methyl (T_6), @ 15% showed minimum disease incidence of 5.3% followed by Difenoconazole(T_4), @ 0.1% gave 7.3 % incidence under *in vivo* condition. (Table 2). The disease incidence recorded in check plot was 42.9 per cent. Maximum yield was also recorded in plot drenched with Mancozeb +Thiophanate Methyl(T_6), (470.9 q/ha) followed by Difenoconazole(T_4), (451.0 q/ha). The minimum yield was recorded in undrenched check plot (349.8 q/ha) under *in vivo* condition. Gupta and Bansal (2003) reported Mancozeb and Thiophanate methyl at 0.2% concentration found significantly effective against *F. oxysporum* causing fenugreek wilt under pot conditions which suppots the present finding. According to Poddar *et al.* (2004), systemic fungicides such as propiconazole, thiophanate methyl, and tebuconazole were effective against *Fusarium oxysporum* in chickpea. Sahu *et al* (2020) also reported that broad spectrum combi-

Table 1. Effect of fungicides on radial growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* under *in vitro* condition

S. No.	Fungicides	Dosage (g/liter)	Radial growth (mm) after 144 hrs*	% growth inhibition over check	Sporulation#
T_1	Azoxystrobin	1.0	15.0	55.2	+++
T_2	Propineb	2.0	10.3	69.2	++
T_3	Thiophanate Methyl	1.0	0.0	100.0	-
T_4	Difenoconazole	1.0	0.0	100.0	-
T_5	Mancozeb	2.0	10.0	70.1	++
T_6	Mancozeb + Thiophanate Methyl	1.5	0.0	100.0	-
T_7	Boscolid+ Pyraclostrobin	1.0	10.3	69.2	+++
T_8	Control Nil	-	33.5	--	++++
	SE(m)		0.061		
	CD(0.05)		1.843		

*Average of three replications; # Excellent: ++++, Good: +++, Fair: ++,Poor: +,Nil: -

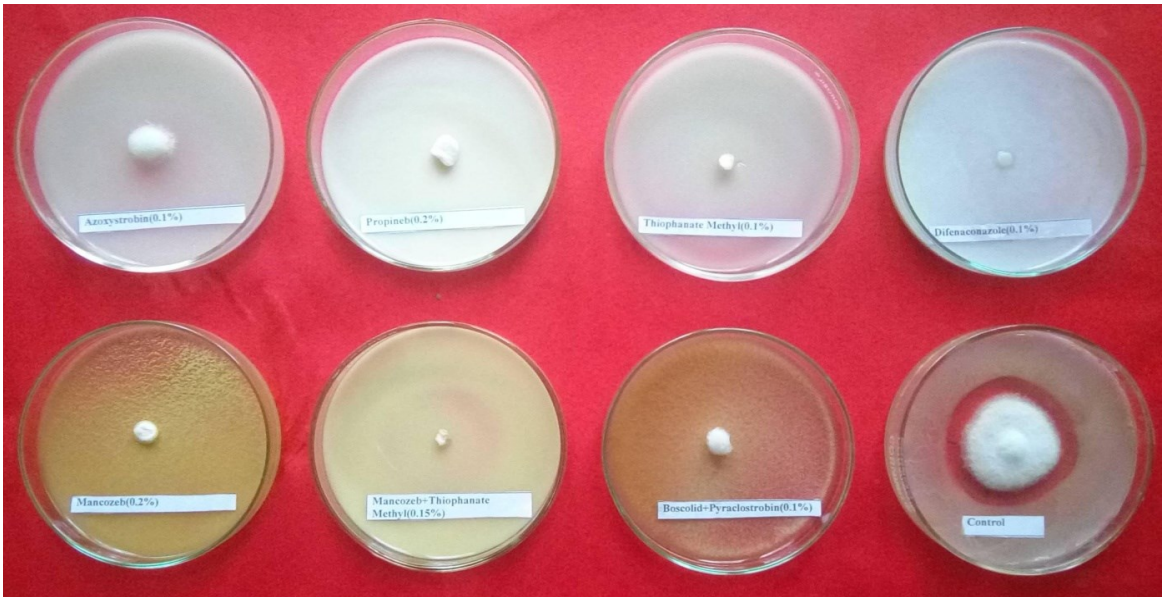


Fig.1. *Effect of fungicides on radial growth of Fusarium oxysporum f. sp. lycopersici ; Azoxystrobin (T_1); Propineb (T_2);Thiophanate Methyl(T_3); Difenoconazole(T_4); Mancozeb(T_5); Mancozeb + Thiophanate Methyl (T_6); Boscolid + Pyraclostobin (T_7); Control (T_8)*

nation of Carbendazim + Mancozeb completely inhibited the growth of *F. oxysporum glycines* followed by Carbendazim (88.74%). Further, two soil drenching of Carbendazim + Mancozeb @ 0.25 per cent was also found to be the best for managing the disease. Systemic fungicides, namely difenoconazole and thiophanate-methyl, have also been effective against tomato wilt.

Evaluation of plant extracts

Gingiber officinalis extract (T_{10}) was found very promising as it produced 82.2 percent growth inhibition of *F. oxysporum* f.sp. *lycopersici* at 15 percent concentration followed by *Allium sativum*(T_{11}) (76.1%) and *Azadirachta indica* leafextract (T_{12}) (73.8%). Other plant extracts produced 27.2 to 57.2 percent growth inhibitions at 15 per cent concentration under *in vitro* condition *(*Table 3*).* An inhibitive effect is proportional to the concentration of ginger rhizome and garlic clove crude extract: the higher the concentration of ginger rhizome, garlic clove crude extract showed the more inhibitive effects. Two soil drenching with *Allium sativum* extracts (T_{11}) @ 15 percent showed minimum disease incidence of (16.6%) with maximum yield (435.3 q/ha) followed by *Gingiber officinalis* extract(T_{10}), (18.4% & 428.2 q/ha) under *in vivo* condition (Table 4). The results are confirmatory with those reported by Ohunakin and Bolanle (2017), who reported that *A. sativum* at 10% concentration gave the highest inhibitory effect (70.24%) on mycelial growth followed by *G. officinalis* which gave 65.92 % inhibition. It was observed that the higher the concentration, the higher the inhibitory effect. Hadian (2012) reported 98% mycelia inhibition of pathogen by *A. indica* seed extract under *in vitro* condition. Kumar *et al(* 2017)reported that root dip treatment of tomato seedlings with extracts of *A. sativum* reduced the wilt by 80 per cent. Sahu *et al.*(2020) also reported

Table 2. Effect of soil drenching of fungicides on disease incidence and yield of Tomatounder *in vivo* conditions

S.No.	Fungicides	Doses (g/liter)	Disease Incidence	Percent inhibition over control	Yield (q/ha)	% increase in yield over check
T_1	Azoxystrobin	1.0	23.3	45.7	398.3	13.8
T_2	Propineb	2.0	29.0	32.4	372.2	06.4
T_3	Thiophanate Methyl	1.0	10.3	76.0	435.3	24.4
T_4	Difenoconazole	1.0	07.3	83.0	451.0	28.9
T_5	Mancozeb	2.0	15.3	64.3	422.0	20.6
T_6	Mancozeb + Thiophanate Methyl	1.5	05.3	87.6	470.9	34.3
T_7	Boscolid + Pyraclostobin	1.0	17.6	59.0	430.0	22.9
T_8	Control	Nil	42.9	--	349.8	
	SE(m)		0.875		3.328	
	CD (0.05)		0.680		10.193	

*Average of three replications

Table 3. Effect of plant extracts on radial growth and sporulation of *Fusarium* under *in vitro* conditions

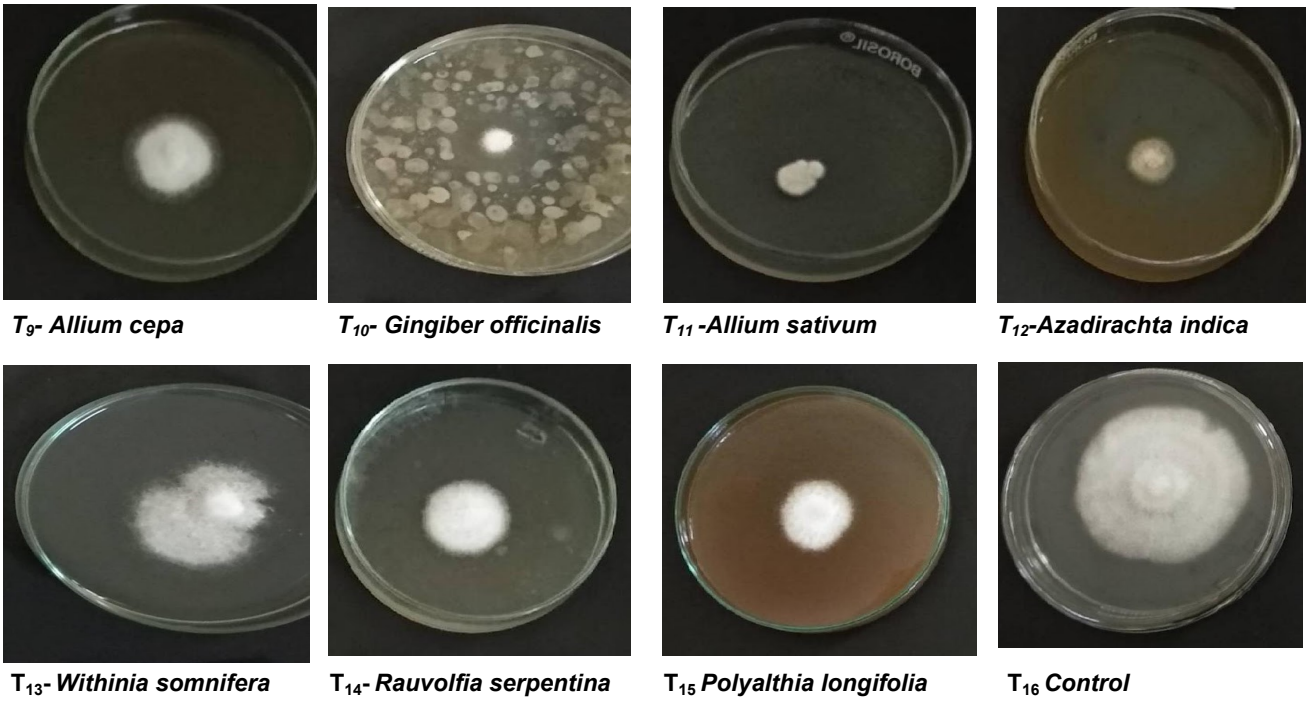
Treatment No.	Name of plant extracts	Local name	Parts used	Radial growth of target pathogen (mm)*			Mean %	% growth inhibition			Sporulation#		
				5%	10%	15%							
T_9	*Allium cepa*	Onion	Bulb	29.8	28.0	18.8	25.5	32.2	36.3	57.2	++++	++++	+++
T_{10}	*Gingiber officinalis*	Ginger	Rhizome	14.0	12.3	7.8	11.4	68.1	72.0	82.2	+++	+++	+
T_{11}	*Allium sativum*	Garlic	Clove	36.6	24.6	10.5	23.9	16.6	44.0	76.1	+++	+++	+
T_{12}	*Azadirachta indica*	Neem	Leaf	39.5	29.6	11.5	26.9	10.2	32.7	73.8	+++	+++	++
T_{13}	*Withania somnifera*	Ashwagandha	Leaf	35.1	35.1	32.0	34.1	20.2	20.2	27.2	++++	++++	++++
T_{14}	*Rauvolfia serpentina*	Sarpagandha	Leaf	21.1	21.1	21.0	21.1	52.0	52.0	52.2	+++	+++	+++
T_{15}	*Polyalthia longifolia*	Ashok	Leaf	33.0	32.5	27.3	30.9	25.0	26.1	37.9	++++	++++	++++
T_{16}	Control			44.0	44.0	44.0	44.0	-	-	-	++++	++++	++++
	SE(m)			0.862	0.945	0.866							
	CD(0.05)			2.607	2.856	2.619							

#- Excellent: ++++, Good: +++, Fair: ++,Poor: +,Nil: -

Table 4. Effect of soil drenched of plant extracts on disease incidence and yield of tomato under *in vivo* condition

Treatment no.	Name of plant extracts	Doses (%)	Disease Incidence (%)	Percent disease control over check (%)	Yield (q/ha)	% increase in yield over check
T_9	*Allium cepa*	15	27.3	32.2	400.3	14.8
T_{10}	*Gingiber officinalis*	15	18.4	54.3	428.2	22.9
T_{11}	*Allium sativum*	15	16.6	58.8	435.3	24.9
T_{12}	*Azadirachta indica*	15	26.0	35.4	398.0	14.2
T_{13}	*Withania somnifera*	15	33.7	16.3	374.0	07.3
T_{14}	*Rauvolfia serpentina*	15	35.0	13.1	368.9	05.9
T_{15}	*Polyalthia longifolia*	15	37.5	06.9	352.0	01.0
T_{16}	Control	Nil--	40.3	--	348.3	--
	SE(m)		0.527		2.853	
	CD (0.05)		1.614		8.737	

*Average of 3 replications



T_9- Allium cepa ***T_{10}- Gingiber officinalis*** ***T_{11} -Allium sativum*** ***T_{12}-Azadirachta indica***

T_{13}- *Withinia somnifera* **T_{14}- *Rauvolfia serpentina*** **T_{15} *Polyalthia longifolia*** **T_{16} *Control***

Fig. 2. *Effect of plant extracts at 15% concentration on radial growth of Fusarium oxysporum f. sp.lycopersici ; Allium cepa(T_9); Gingiber officinalis (T_{10}); Allium sativum(T_{11}); Azadirachta indica(T_{12}); Withania somnifera (T_{13}); Rauvolfia serpentina (T_{14}),, Polyalthia longifolia(T_{15}); Control (T_{16})*

that garlic clove extract *in vitro and in vivo* @15 per found best as it produced 73.00, per cent growth inhibition of *F. oxysporumglycines*. The two soil drenching of garlic extracts at 15.0 per cent was found the best for managing Fusarium wilt of soybean in field condition. Awad (2016) also reported that garlic clove extract at a concentration of 5% was most effective in controlling the sudden wilting of the watermelon plant under *in vivo* conditions. These plants are widely used because of their importance in traditional medicine and their high amounts of polyphenols, flavonoids, phenolic acids, tannins, quinines, coumarins, terpenoids, and alkaloids (Farheen *et al.,* 2005; Omidbeygi *et al.,* 2007; Oyedeji *et al.,* 2011).

Mechanisms putatively responsible for pathogen toxicity include disruption of cell wall synthesis, alteration of cell permeability, disruption of electron transport, nutrient absorption, adenosine triphosphatase and other cellular metabolic processes, and inactivation of various cellular enzymes, transformation, and denaturation of intracellular proteins (Feng and Zheng, 2007; Al-Amiery, 2012). Flavonoids, phytic acid, tannins and phenols are all found in *A. sativum*. Its aqueous extract almost completely inhibited the mycelium of Fusarium (Salem *et al*., 2021).

Conclusion

It was concluded that Fusarium wilt is one of the prevalent diseases of tomatoes in Madhya Pradesh. Regarding its management, the fungicides were found to be more effective compared to plant extracts. The Mancozeb + Thiophanate Methyl and Difenoconazole fungicides were the most effective, followed by *G. officinalis* and *A. sativum* extracts at their higher concentrations.

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Conflict of interest

The authors declare that they have no conflict of interest.

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