

Original Research Article

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Invitro Evaluation of Botanicals and ITKs against *Septoria lycopersici* Causing Septoria Leaf Spot of Tomato

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the commercially important major vegetable crops grown throughout the tropics and subtropics. Septoria leaf spot caused by *Septoria lycopersici* Sp. is one of the most destructive diseases of tomato and is particularly severe in areas where wet, humid weather persists for extended periods. The bio assay of botanicals and Indigenous technical knowledge (ITKs) forms the prerequisite for the field evaluation. In the present investigation eight botanicals and eight ITKs were evaluated at 5, 10 and 15 per cent concentration against *S. lycopersici* under laboratory condition. Among the eight botanicals, *Allium sativum* (Garlic) bulb extract found to be significantly superior over other treatments in inhibiting the mycelial growth (65.10 %) of *S. lycopersici* followed by *Duranta goldiana* (Duranta) leaf extract (53.73 %) and lowest inhibition was observed in *Tagetes erecta* (Marigold) leaf extract (23.53 %) at 15 per cent concentration. *In vitro* spore germination bioassay of ITKs revealed that, highest inhibition (76.67 %) of conidial germination over control was shown by Cow urine which was on par with Panchagavya (73.33 %) and the least inhibition was observed in biodigestor (33.33 %) at 15 per cent concentration.

Keywords

Tomato, *Septoria lycopersici*, *In vitro*, Botanicals, ITKs

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Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most remunerative and widely grown vegetables in the world. It is cultivating extensively for its edible fruits, which are rich source of vitamin A and C. Fruit is bright red due to the pigment lycopene, which is the major carotenoid in tomatoes, exhibits the highest antioxidant activity and it is a

beneficial colouring agent for food because of its solid color and nontoxicity (Schulzova and Hajslova, 2007).

In India, the crop is being cultivated in a variety of climate, across states of Maharashtra, Andhra Pradesh, Karnataka, West Bengal, Odisha, Bihar, Madhya Pradesh, Uttar Pradesh, Gujarat and Chhattisgarh.

Tomato production is hampered by a variety of plant pathogens such as bacterial, fungal, nematode and viral diseases and also by abiotic factors (Blancard *et al.*, 1992). Fungal diseases like damping-off, late blight, early blight, powdery mildew, fusarium wilt, grey leaf spot, anthracnose, septoria leaf spot, buckeye rot (Sokhi *et al.*, 1991) are the major biotic threats for tomato cultivation.

Among the fungal foliar diseases, Septoria leaf spot (*Septoria lycopersici* Speg.) of tomato is one of the devastating diseases of tomato causing yield loss up to 12 to 16 per cent (Rizinski, 1966). Extensive periods of more relative humidity, leaf wetness and temperatures of 20 to 25°C are favourable environments for progress and spread of the pathogen (Weber, 1982; Elmer and Ferrandino, 1995). Symptoms generally include circular or angular lesions of about 1.5 to 6 mm in width with gray centers, dark brown margins and are most commonly found on the older, lower leaves of the plant. Small pycnidium fruiting bodies can be seen dispersed on the spots with rare infection on fruits.

Although, satisfactory control of the disease by using various chemicals has been documented (Choulwar and Datar, 1988; Maheshwari *et al.*, 1991), the continuous use of these agrochemicals for controlling the disease may pose several problems like toxicity to non-target organisms, development of resistance in the populations of the pathogen and environmental pollution.

Use of botanicals is a cost effective, ecofriendly measure in plant disease management because they are easily biodegradable and plants synthesize aromatic secondary metabolite like phenolic acids, phenols, flavones, flavonols, flavonoids, quinones, coumarins and tannins and these compounds serve as plant defence

mechanisms against pathogenic microorganisms (Cowan, 1999). Another environmentally safe practice in plant disease management is the use of ITKs (Indigenous technical knowledge) such as use of cow urine, cow dung, panchagavya, jeevamruta, vermiwash *etc.*, which are reported to have antifungal activity against the pathogens (Raut and Vaidya, 2018). Hence, the use of the botanicals and ITKs needs more research as an eco-friendly measure for managing the fungal diseases of plants which can even be used as one of the components in integrated disease management.

Materials and Methods

Evaluation of botanicals

Fresh leaves of *Pongamia pinnata*, *Duranta goldiana*, *Bougainvillea glabra*, *Eucalyptus globule*, *Tagetes erecta* and fresh bulbs of *Allium sativum*, *Allium cepa* were collected from different locations of Dharwad. Hundred grams of the fresh leaves/bulbs were washed with water, air dried and crushed in a mortar and pestle by adding sterile distilled water at the rate of 100 ml/100g of plant tissue and homogenized in an electric blender. The homogenate was filtered out through double layer of muslin cloth and centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and filtered through Whitman number 1 filter paper to avoid microbial contamination.

For preparation of Neem seed kernel extract, dried neem seeds were taken and washed thoroughly and then air dried. Known quantity of kernel (100g) were weighed, grinded the kernels gently to powder it and then soaked in same quantity (100ml) of water for 24 h. Stirred and filtered twice with double layer of muslin cloth, centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and filtered through Whitman number 1 filter paper.

The resultant extracts were considered as 100 per cent concentration (Stock solution). Later, from that stock solution, working solutions of required concentration were made. These extracts were evaluated at three concentrations (5, 10, and 15 %) using poison food technique (Shravelle, 1961) against *S. lycopersici*.

Potato dextrose agar was used as nutrient medium and required quantity of each plant extract was added separately so as to get a requisite concentration of the plant extract. 20 ml of poisoned medium was poured to each of the 85 mm petri dishes and allowed for solidification.

The actively growing hyphal bit (5 mm) from periphery of the seven day old culture of *S. lycopersici* was carefully cut using a cork borer and transferred aseptically to the centre of each petri dish containing the poisoned medium. Suitable control was maintained by growing the cultures on PDA without the plant extract. The plates were incubated at $27 \pm 1^{\circ}\text{C}$ for seven days and the colony diameter was recorded.

Per cent inhibition over control was worked out according to formula given by Vincent (1947).

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

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

Evaluation of ITKs

ITKs viz; jeevamrutha, panchgavya, vermiwash, biodigestor, buttermilk, cow urine, turmeric and ginger was collected from the bioresource farming unit, Institute of Organic Farming, UAS, Dharwad and used

for laboratory studies. These products were evaluated *in vitro* against the pathogen at 5, 10 and 15 per cent concentrations against *S. lycopersici*. To study the antifungal activity of ITKs and also the effective concentration, cavity slide method was followed as suggested by Nene and Thapliyal (1982).

Working solutions of 5, 10 and 15 per cent concentrations were made from the stock solution with sterile distilled water and these concentrations were tested *in vitro* on the germination of conidia of *S. lycopersici*. Each ITKs concentration was replicated three times. Germination of conidia was recorded after 24 h of incubation in moist chamber at room temperature. Spores incubated in sterile distilled water served as control. Per cent inhibition over control was calculated by using the formula given by Vincent (1947).

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

Where, I = Per cent inhibition of spore germination, C = Germination of spores in control

T = Germination of spore in treatment

Results and Discussion

Evaluation of botanicals

Present experiment was conducted to know the antifungal action of eight botanicals at three different concentrations 5, 10 and 15 per cent against *S. lycopersici* by using poison food technique as stated in Material and Methods.

The results revealed that, among the three concentrations tested, all the treatments recorded significantly higher inhibition of the pathogen at 15 per cent concentration followed by 10 per cent and 5 per cent

concentrations (Plate 1). Among the eight botanicals tested against *S. lycopersici* highest per cent growth inhibition of mycelium was exhibited by *Allium sativum* bulb extract (65.10%) was significantly superior over other treatments followed by *Duranta goldiana* leaf extract (53.73%), *Allium cepa* bulb extract (51.76%), *Eucalyptus globule* (50.59%), *Pongamia pinnata* (47.84%), Neem seed kernel extract (41.57%), *Bougainvillea glabra*(36.08%) and lowest inhibition was observed in *Tagates erecta* leaf extract (23.53%) at 15 per cent concentration. The results are presented in Table 1 and Figure 1.

The results are in accordance with Lal, M. *et al.*, (2016) found that, Neem (*Azadirachta indica*) was most effective in in inhibiting the fungal growth (47.13 %) of *S. lycopersici*

followed by Ginger (*Zingiber officinale*) 45.98 per cent, garlic (*Allium sativum* L.) 43.68 per cent and onion (*Allium cepa*L.) 41.37 per cent.

Pankaj and Singh (2017) reported that, the *Allium sativum* extract was one of the effective botanical for management of early blight (*A. solani*). Roopa *et al.*, 2014, reported that garlic (37.04%), *duranta* (34.44%) and neem leaf extract (32.41%) at 10 per cent were effective in inhibiting the mycelial growth of *A. solani*. Garlic bulb extract is known to have antifungal activity against *C. capsici*(Murthy and Amonker, 1974). Singh *et al.*, (1990) reported that a compound ajoene, derived from garlic inhibited *Colletotrichum* spp.

Table.1 *In vitro* evaluation botanicals against *Septoria lycopersici*

Treatments	Botanicals	Per cent inhibition of mycelial growth			
		5%	10%	15%	Mean
T ₁	<i>Allium sativum</i>	26.27 (30.84)*	49.41 (44.66)	65.10 (53.79)	46.93 (43.24)
T ₂	<i>Duranta goldiana</i>	25.10 (30.06)	42.35 (40.60)	53.73 (47.14)	40.39 (39.46)
T ₃	<i>Allium cepa</i>	20.78 (27.13)	40.39 (39.46)	51.76 (46.01)	37.65 (37.85)
T ₄	<i>Eucalyptus globule</i>	18.82 (25.71)	34.90 (36.21)	50.59 (45.34)	34.77 (36.13)
T ₅	<i>Pongamia pinnata</i>	14.90 (22.71)	31.76 (34.31)	47.84 (43.76)	31.50 (34.14)
T ₆	Neemseed kernel extract (NSKE)	13.73 (21.75)	25.49 (30.32)	41.57 (40.15)	26.93 (31.26)
T ₇	<i>Bougainvillea glabra</i>	12.16 (20.41)	20.00 (26.56)	36.08 (36.92)	22.75 (28.48)
T ₈	<i>Tagates erecta</i>	6.27 (14.51)	14.51 (22.39)	23.53 (29.02)	14.77 (22.60)
	Mean	17.25 (24.54)	32.35 (34.66)	46.27 (42.86)	
		S.Em. ±		C.D. @ 1 %	
Treatment (T)		1.30		3.92	
Concentration (C)		0.79		2.37	
T x C		2.20		6.60	

* Arc sine transformed values

Table.2 *In vitro* evaluation ITKs against *Septoria lycopersici*

Treatments	ITKs	Per cent inhibition of spore germination			
		5%	10%	15%	Mean
T ₁	Cow urine	56.67 (48.83)*	63.33 (52.73)	76.67 (61.12)	65.56 (54.06)
T ₂	Panchagavya	50.00 (45.00)	60.00 (50.77)	73.33 (58.90)	61.11 (51.42)
T ₃	Jeevamrutha	43.33 (41.17)	56.67 (48.83)	66.67 (54.74)	55.56 (48.19)
T ₄	Vermiwash	40.00 (39.23)	53.33 (46.91)	60.00 (50.77)	51.11 (45.64)
T ₅	Turmeric powder	36.67 (37.26)	40.00 (39.23)	53.33 (46.91)	43.33 (41.17)
T ₆	Buttermilk	33.33 (35.26)	36.67 (37.27)	46.67 (43.09)	38.89 (38.59)
T ₇	Ginger rhizome extract	26.67 (31.09)	33.33 (35.26)	43.33 (41.17)	34.44 (35.94)
T ₈	Biodigestor	20.00 (26.56)	26.67 (31.09)	33.33 (35.26)	26.67 (31.09)
	Mean	38.33 (38.25)	46.25 (42.85)	56.67 (48.83)	
		S.Em. ±		C.D. @ 1 %	
Treatment (T)		1.24		3.72	
Concentration (C)		0.76		2.28	
T x C		2.15		6.45	

* Arc sine transformed values

Evaluation of ITKs

Eight ITKs were used to test their efficacy in inhibiting the conidial germination of *S. lycopersici* by using spore germination technique as detailed in Material and Methods. Among the three concentrations tested, the maximum inhibition of conidial germination was observed at 15 per cent concentration followed by 10 per cent and 5 per cent concentrations. Out of the eight ITKs tested against *S. lycopersici*, cow urine (76.67

%) at 15 per cent concentration was found to be superior in inhibiting germination of conidia of *S. lycopersici* which was on par with panchagavya (73.33 %) followed by jeevamrutha (66.67 %), vermiwash (60.00%), turmeric powder (53.33%), buttermilk (46.67%), ginger rhizome extract (43.33%) and the least inhibition was observed in biodigestor (33.33 %) at 15 % concentration. Results from this experiment are presented in the Table 2, Plate 2 and Figure 2.

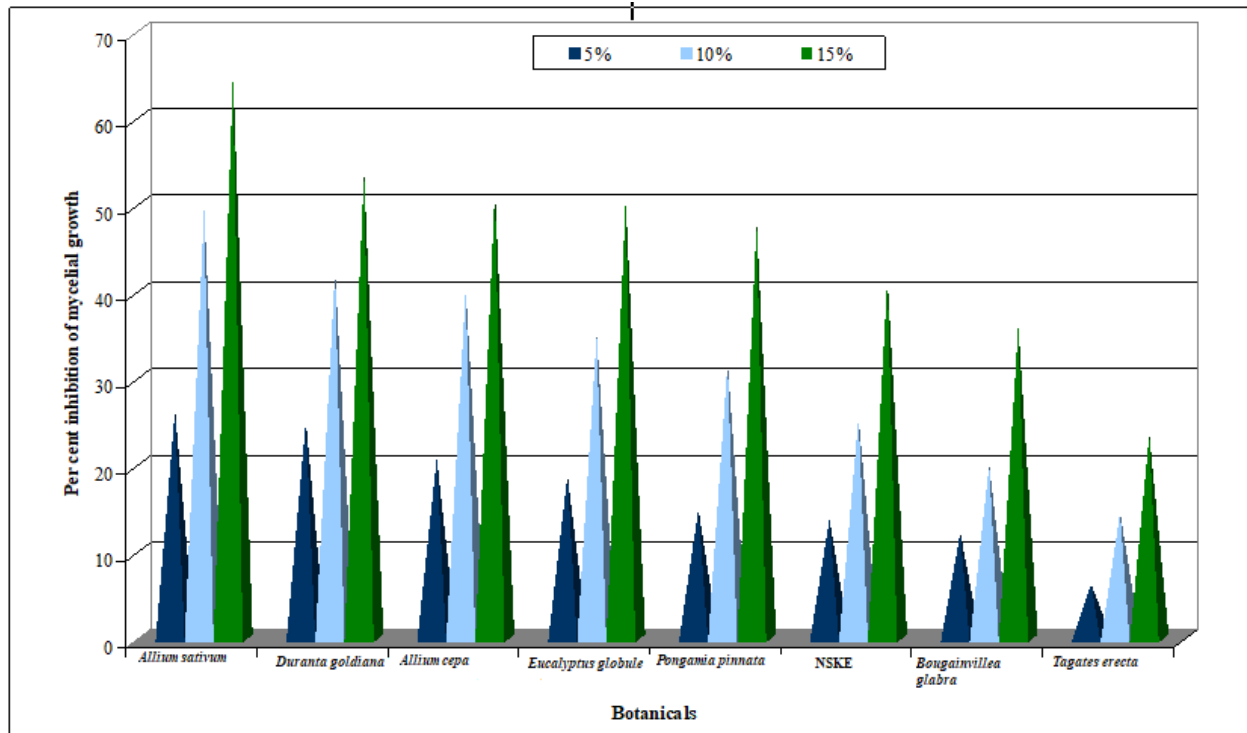


Fig.1 In Vitro evaluation of botanicals against *Septoria Lycopersici*

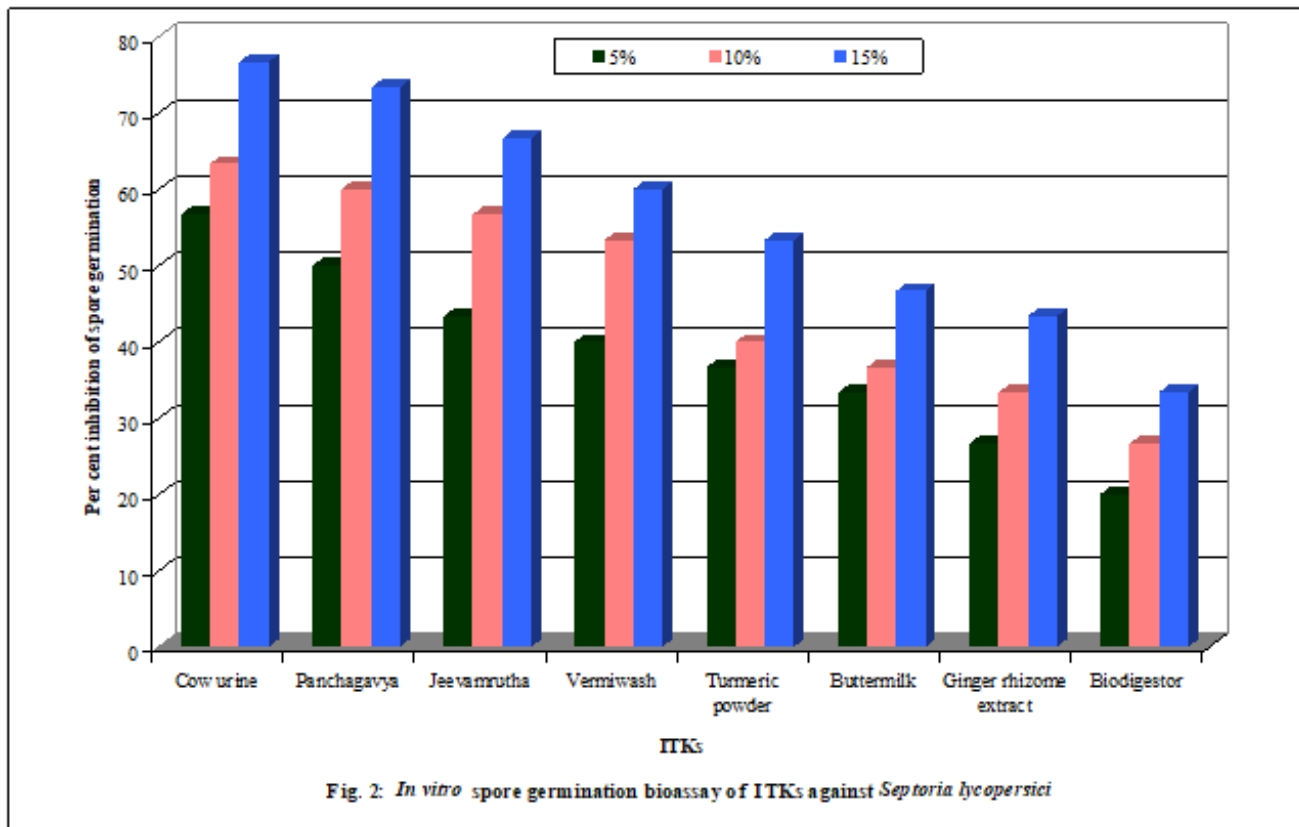
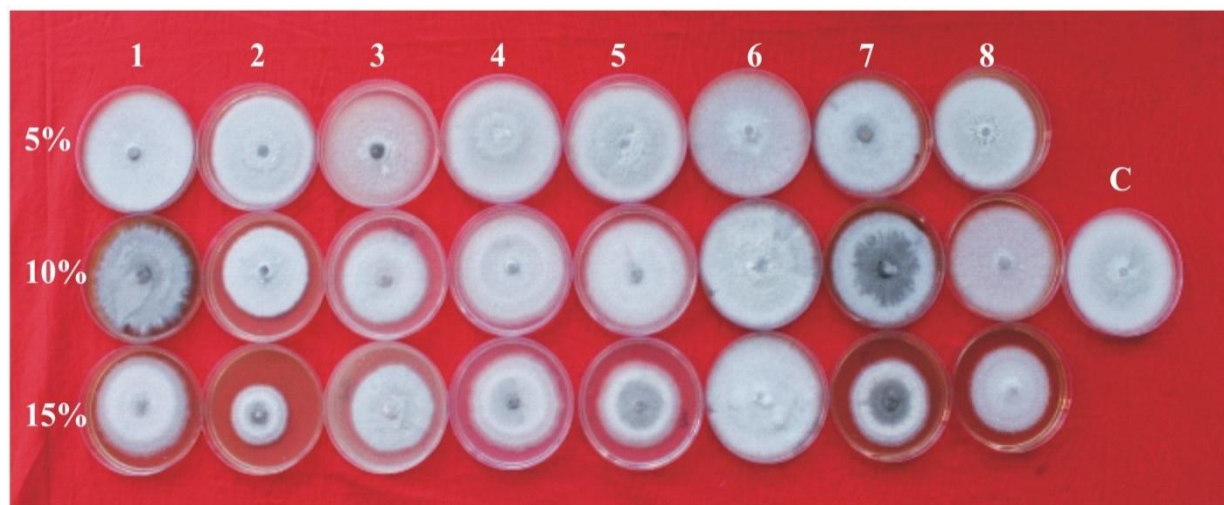


Fig. 2: In vitro spore germination bioassay of ITKs against *Septoria lycopersici*

Fig.2 In Vitro spore germination bioassay of ITKs against *Septoria Lycopersici*



1. *Bougainvillea glabra*, 2. *Allium sativum*, 3. *Duranta goldiana* 4. NSKE 5. *Allium cepa*
6. *Tagetes erecta* 7. *Eucalyptus globule* 8. *Pongamia pinnata* 9. Control

Plate.1 *In vitro* evaluation of botanicals against *Septoria lycopersici*



a) Cow urine @ 15%

b) Untreated control

Plate.2 *In vitro* spore germination bioassay of ITKs against *Septoria lycopersici*

The results are in agreement with Savita *et al.*, (2015) who found that cow urine at 15 per cent concentration was most effective against three fungal pathogens (*Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*) of methi and bhendi. Basak *et al.*, (2005) found that cow urine was very effective in controlling wilt disease of cucumber caused by *Fusarium oxysporum* f. sp. *Cucumerinum* and *Fusarium solani* f.sp. *cucurbitae*. Patel *et al.*, (2015) found that cow urine was best against red rot disease of sugarcane *Colletotrichum falcatum*. The antifungal

activity of cow urine might be due to the presence of dimethylamine, inorganic phosphorus. Janadik and Sharma (2016) reported that cow urine has magnificent antifungal power, growth enhancing and germicidal influence on plant pathogens. Cow urine is shown experimentally to possess various biological activities such as antidiabetic (Gururaja *et al.*, 2011), antimicrobial (Upadhyay *et al.*, 2010), antioxidant (Krishnamurthi *et al.*, 2004), antitumor (Raja and Agarwal, 2010), molluscicidal (Kumar *et al.*, 2011) and other.

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