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Efficacy of different botanicals against the *Alternaria solani* under *in vitro* conditions

Deshmukh HV, Deokar CD, Khaire PB and Brahmane PR

Abstract
Among the six-plant extract tested, most effective plant extract was found *Allium sativum* (Garlic) which exhibited minimum mycelium growth (21.60 mm) at 15% concentration with the mean average minimum mycelium growth (52.86 mm). It was significantly lower of over rest of the plant extracts. However, maximum mean average mycelium growth (80.53 mm) was observed in *Azadirachta indica* (Neem). In case of concentrations, minimum growth of the mycelium was observed in higher concentration (15%) in all the plant extracts. It's indicated that the mycelium growth was reduced with gradually increased in concentration of plant extract. Interaction was also found significant. In case of interaction between plant extract and concentration, mean minimum mycelium growth (52.86 mm) was found in *Allium sativum* (Garlic) which was at par with Jatropha curcas (Jatropha) (53.63 mm) and significantly lower over rest of plant extracts.

Keywords: Botanicals, *Alternaria solani*, mycelia growth, inhibition

Introduction
Tomato (*Lycopersicon esculentum* Mill.) widely grown vegetable in the world and it is the second most important remunerable solanaceous vegetable crop after potato (Sahu *et al.*, 2013). Tomato is a model species for classical genetic and genomic studies. Tomato has high medicinal value. It acts as a promoter of gastric secretion and blood purifier. It is popular because it supplies vitamin C and adds variety of colour and flavour to food.

Tomato suffers with various diseases cited by fungi, bacteria, viruses, nematodes etc. in several countries (Mark *et al.*, 2006) [18]. More than 200 diseases have been reported to infect tomato in the world (Atherton and Rudich, 1986) [2]. Large number of fungal diseases such as early blight (*Alternaria solani*), Late blight (*Phytophthora infestans*), Septoria leaf blight (*Septoria lycopersici*), Powdery mildew (*Oidiopsistaurica*), Fusarium wilt (*Fusarium oxysporum f. sp. lycopersici*), Collar rot (*Sclerotium rolfsii*), and Damping off (*Pythium sp*.) are causes severe losses in tomato. Among the fungal diseases, early blight caused by *Alternaria solani* is one of the most important and frequent occurring disease of the crop nation and worldwide (Jones *et al.*, 1991) [10].

Genus *Alternaria* belongs to deuteromycetes having different species, which are destructive plant pathogen to the families such as Solanaceae, cucurbitaceae, brasicaceae. Species of the *Alternaria* genus are cosmopolitan, surviving both as saprophytes as well as weak parasites. In several cases, small dark coloured spots are also formed on pods and tender twigs (Valkonen and Koponen, 1990) [27]. Diseases caused by *Alternaria* are among the most common disease of many kinds of plants throughout the world. Total aggregate losses caused by the various *Alternaria* on all of their hosts rank among the highest caused by any pathogen (Agrios, 2005) [1]. A comprehensive, comparative account of morphological differentiation of different *Alternaria species* occurring on cucurbitaceous, brassicaceous and solanaceous crops are described by Khalid *et al.* (2004) [16] and Deshwal (2004) [5].

Alternaria species are foliar pathogens that cause a relatively slow destruction of host tissues through the reduction of photosynthetic potential. An infection leads to the formation of necrotic lesions which sometimes have a target board like appearance due to growth interruptions caused by unfavourable conditions. The fungus resides in the centre of the lesion, which is surrounded by an un-invaded chlorotic halo, a symptom that is commonly observed for the infection process of necrotrophic pathogens. Members of the genus *Alternaria* frequently cause quiescent infections in which the fungus enters the tissue where it remains dormant until changed conditions favour infection. *Alternaria* has no known sexual stage or over wintering spores, but the fungus can survive as mycelium or spores on decaying plant debris for a considerable time, or as a latent infection in seeds (Rotem, 1994).

Plant extracts are considered as new rays of hope because they are eco-friendly and can be used as an effective alternative measure to control plant diseases. Hence, the present investigation has been planned to develop eco-friendly management of the disease using botanicals eco-friendly management option will help in reducing residues of fungicides as well as environmental pollution.

Material and Methods

In vitro evaluation of botanicals

Efficacy of botanicals (plant extracts) against *Alternaria solani* were tested by using poisoned food technique under *in vitro* conditions (Nene and Thapliyal, 1993). Fresh healthy plant parts of 100 g (leaves/rhizomes/cloves) each, as listed in Table No. 1 were collected from field, then they were washed with tap water and air dried. Then crushed separately in 100 ml. of distilled water (w/v). Macerates obtained were filtered separately through double layered muslin cloth in 100 ml. volumetric flask (100 ml cap.).

Potato dextrose agar medium was used as nutrient medium and required quantity of each plant extract was added separately to get a required concentration of the plant extract. The plant extract was thoroughly mixed with PDA medium and sterilized at 121 ^{0}C for 20 minutes. Twenty milliliters of poisoned medium were poured to each of the 90 mm petri dishes and allowed for solidification. Simultaneously without plant extract PDA was poured in petri dishes as control. Actively growing 7-day old culture of *A. solani* was carefully cut using a cork borer and transferred aseptically at the center of each petri dish containing the poisoned/non-poison solid medium. The plates were incubated at 25 ± 2 ^{0}C. Each treatment was replicated four times. The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the equation (Vincent, 1947). Details of treatments are given below:

Treatment details

Table 1: Treatment details of botanicals

Treatment	Treatment Details	Local name	Botanical name	Plant parts used
T_1	Neem Extract	Neem	*Azadirachta indica*	Leaves
T_2	Garlic Extract	Garlic	*Allium sativum*	Bulb (cloves)
T_3	Ginger extract	Ginger	*Zingiber officinale*	Rhizome
T_4	Ghaneri extract	Ghaneri	*Lantena camera*	Leaves
T_5	Jatropha extract	Jatropha	*Jatropha curcas*	Leaves
T6	Datura extract	Datura	*Datura stramonium*	Leaves
T_7	Control	---	---	---

Concentrations: 5, 10 and 15 per cent, Replications: Four, Design: CRD

Observation recorded

The radial growth of the fungus on the poisoned medium was recorded at time of mycelium growth reached 90 mm in control. Per cent inhibition of mycelium growth of the fungus was calculated by using the formula described by Vincent (1947a)

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

Where,

I = Per cent inhibition
C = Radial growth in control
T= Radial growth in treated (fungicide/ botanicals/ bioagents).

Result and Discussion

Evaluation of Botanicals against *Alternaria solani*

Data regarding on *In vitro* efficacy of plant extracts against *Alternaria solani* have been presented in Table 2 indicated that significant difference on mycelium growth was recorded in different plant extract in at all the concentrations. Among the six-plant extract tested, most effective plant extract was found *Allium sativum* (Garlic) which exhibited minimum mycelium growth (21.60 mm) at 15% concentration with the mean average minimum mycelium growth (52.86 mm). It was significantly lower of over rest of the plant extracts. However, maximum mean average mycelium growth (80.53 mm) was observed in *Azadirachta indica* (Neem). In case of concentrations, minimum growth of the mycelium was observed in higher concentration (15%) in all the plant extracts. It's indicated that the mycelium growth was reduced with gradually increased in concentration of plant extract (Plate 1). Interaction was found significant. In case of interaction between plant extract and concentration, mean minimum mycelium growth (52.86 mm) was found in *Allium sativum* (Garlic) which was at par with *Jatropha curcas* (Jatropha) (53.63 mm) and significantly lower over rest of plant extracts. On the other hand, maximum inhibition in mycelium growth was noticed in *Allium sativum* (Garlic) @ 15% (76.00%) followed by *Jatropha curcas* (Jatropha) @ 15% (69.33%), while *Jatropha curcas* (Jatropha) @ 10% (32.22%) fallowed by *Datura stramonium* (Datura) @ 10% (28.88%) and *Datura stramonium* (Datura) @ 5% (26.33%) followed by *Allium sativum* (Garlic) @ 5% (22.22%), while minimum inhibition in mycelium growth was recorded, 13.33% @ 15%, 9.66% @ 10% and 8.55% @ 5% in *Azardirechta indica* (Neem). The present findings are confirmed with the results of Wszelaki and Miller (2005) they reported that garlic extracts significantly reduce the leaf blight disease on tomato.

Panchal *et al.* (2009) [21] are reported significantly the lowest mycelial growth of *A. alternata In vitro* was in medium containing garlic clove extracts (10%). Gachande (2010) [7] extracts of 15 plant parts were tested against spore germination and mycelial growth of *Alternaria solani* isolates. The extracts of *Allium sativum* was found to be most effective in regulating the growth of fungus. Chethana *et al.* (2012) [4] evaluated bio-efficacy of six plant products against the purple blotch disease of onion caused by *Alternaria porri (Ellis.)* Cif. Among the six plant products evaluated, fresh aqueous extract of garlic (20%) was effective and found 100% inhibition of mycelial growth. Neem oil and Pongamia oil (20%) was found 76.94 and 59.94 per cent inhibition of mycelial growth. Roopa *et al.* (2014) [24] tested bio-efficacy of ten botanicals against *Alternaria solani* casing early blight of tomato. Among the ten plant botanicals evaluated, Jatropa leaf extract @ 10 per cent was found most effective in inhibiting the mycelial growth of *A. solani* (62.78%).

Table 2: *In vitro* evaluation of different botanicals against *Alternaria solani*

Treatment No.	Treatment Details (Botanicals)	Average mycelial growth (mm)*			Mean of Average mycelial growth (mm)	Per cent inhibition of mycelial growth over control (%)			Mean% Inhibition over control
		5%	10%	15%		5%	10%	15%	
T_1	Neem	82.30	81.30	78.00	80.53	8.55 (17.00)	9.66 (18.11)	13.33 (21.41)	10.51 (18.92)
T_2	Garlic	70.00	67.00	21.60	52.86	22.22 (28.12)	25.55 (30.36)	76.00 (60.67)	41.25 (39.96)
T_3	Ginger	81.60	65.00	53.30	66.33	9.33 (17.79)	27.77 (31.80)	40.77 (39.68)	25.95 (30.62)
T_4	Ghaneri	75.60	72.00	32.00	59.86	16.00 (23.58)	20.00 (26.57)	64.44 (53.39)	33.48 (35.35)
T_5	Jatropha	72.30	61.00	27.60	53.63	19.66 (26.32)	32.22 (34.58)	69.33 (56.37)	40.40 (39.47)
T_6	Datura	66.30	64.00	49.30	59.86	26.33 (30.87)	28.88 (32.51)	45.22 (42.26)	33.47 (35.35)
T_7	Control (Untreated)	90.00	90.00	90.00	90.00	00.00 (0.00)	00.00 (0.00)	00.00 (0.00)	00.00 (0.00)
					S.Em. +	0.78	0.87	0.95	
					CD at 5%	2.30	2.56	2.80	
					CV (%)	2.03	2.43	3.78	

CD at 5% level of significance.
*Average of four replications.
Figures in parenthesis (s) are angular transformed value.

Similar results were also reported by Kadam *et al.* (2018) [15] he evaluated the bioagents and botanicals *In vitro* against the *Alternaria alternata*. Among the eleven botanicals tested, significantly highest average mycelial growth inhibition was recorded with *A. sativum* (75.56%), followed by *Z. officinale* (73.64%), *A. indica* (71.17%). Sandipan *et al.* (2014) [26] and Barros *et al.* (1995) [3] found that garlic extract effectively inhibited the growth of *A. alternata* at 250 ppm and reduced colony diameter.

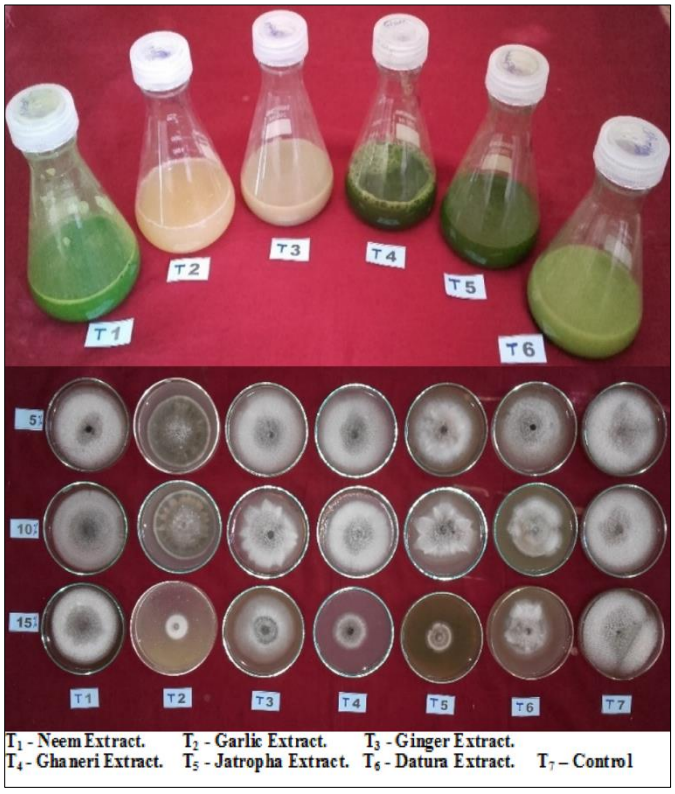

Plate 1: *In vitro* evaluation of different botanicals against *A. solani.*

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