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Antifungal activity of plant extracts against *Fusarium solani* (Mart.) Sacc. *in vitro*

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Abstract

Pointed gourd is an important vegetable crop during lean period. It is affected by various diseases, among them fusarium wilt is one of the major destroyer diseases. Wilt is major threat for pointed gourd cultivation in south Gujarat. It is a cosmopolitan soil borne fungus with both saprophytic and pathogenic members. For the management of this disease, *in vitro* study was conducted to find out most effective phyto-extracts to control disease caused by *Fusarium solani* (Mart.) Sacc.. The poison food technique was employed for the evaluation of antifungal activity of the extracts. The phyto-extracts of commonly available six different plant species viz., NSKE (*Azadirachta indica*), Onion (*Allium cepa*), Garlic (*Allium sativum*), Neem (*Azadirachta indica*), Ginger (*Zingiber officinalis*) and Mint (*Mentha arvensis*). Among all the tested phyto extracts, leaf extract of neem (*Azadirachta indica*) was found significantly most effective with highest mycelial growth inhibition (42.62%), which was found at par with NSKE (41.30%) followed by onion (*Allium cepa*), mint (*Mentha arvensis*) and ginger (*Zingiber officinalis*) to inhibit the mycelial growth of *F. solani*. The highest colony growth (62.27 mm) and lowest percent inhibition (12.96%) was observed in garlic. From this experiment, it was very clear that extract of onion (*Allium cepa*), garlic (*Allium sativum*), neem (*Azadirachta indica*), ginger (*Zingiber officinalis*), mint (*Mentha arvensis*) and NSKE may have some strong toxic principle presents which directly affect the growth of the *F. solani*. This study indicates that the botanical extracts could be a good alternative in developing a potent plant based fungicides which can be used in organic farming for the management of pointed gourd wilt.

Keywords: Antifungal, plant, *Fusarium solani*, *Azadirachta indica*

Introduction

Pointed gourd (*Trichosanthes dioica* Roxb.) is one of the most important cucurbit vegetables in Asian tropical and subtropical regions of the world. It is an annual or perennial herbaceous vine commonly known as pointed gourd (English), Putulika (Sanskrit), Parval (Hindi), Patola (Gujarati) and Patol (Bengali). Pointed gourd is considered to be a poor man's vegetable. Its fruit is good for curing heart and brain disorders, has diuretic and laxative properties and is also cardio tonic. It is recommended against bronchitis, biliousness, high fever and nervousness. It controls blood cholesterol and sugar, weight loss, treats constipation, cures blood and skin diseases, reduces flu and has anti-aging properties (Kumar, 2011) [7]. It is also a great source of calcium which is necessary for the health of the bones. In the ancient Indian medicine system of ayurveda, pointed gourd is used to treat many ailments including gastric problems. It is extensively cultivated in Eastern Indian states particularly in Bihar, Eastern Uttar Pradesh, West Bengal, Assam, Tripura and to some extent in Orissa (Khare, 2004) [6]. It is also grown in Madhya Pradesh, Maharashtra and Gujarat. It is an important crop of South Gujarat particularly in Olpad, Mahuva and Chikhli block. This crop is widely accepted and is available for nearly eight to ten months of a year.

Pointed gourd performs best in a warm and humid climate where rains are abundant. High humidity favours growth and fruit development. Temperatures between 30 °C and 35 °C are considered optimum for growth and fruiting, while temperatures below 20 °C restrict growth. Fresh vines used for field planting should have 8–10 nodes per cutting and should be partially or fully defoliated to check transpiration. The distance between plants is kept between 1.5–2.0 m × 1.5–2.0 m depending on the method of training of vines. A female: male ratio of 9:1 is optimum for ensuring maximum fruit set. Pointed gourd prefers a well-drained sandy loam soil with good fertility (Anon., 2021) [1].

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Fusarium wilt is one of the major destroyer diseases. Wilts are a major threat for agriculture. It is a cosmopolitan soil borne fungus with both saprophytic and pathogenic members. Looking to the importance of this disease and losses caused every year in South Gujarat, this problem is selected. Wilt of pointed gourd is a serious fungal disease caused by *Fusarium solani* (Mart.) Sacc. Typical symptoms of wilt disease in pointed gourd include necrotic lesions, vascular wilt and roots wilt, which induce serious yield losses. To control fusarium wilt disease, different strategies are currently being employed in agriculture. One of these is use of biological agents, use of botanicals and organic amendments are an effective and sustainable alternative approach to control the growth and reproduction of fusarium pathogen. Here we are going to assess the antifungal activity of plant extracts on the growth of *Fusarium solani*.

Materials and Methods

Evaluation of different phyto-extracts were studied *in vitro* by following "Poison Food Technique" against *Fusarium* sp. (Schmitz, 1930) [10]. Fresh healthy plant parts (leaves or cloves of prospective bio-control sources) were collected from fields. Leaves of plants were separately washed two-three times in tap water then in distilled water and allowed to dry at room temperature (25±1 °C) for six hours. Prior to extraction, leaves of each plant (100 g) were crushed separately with 100 ml sterilized distilled water except for cloves of *Allium sativum* which was crushed in acetone which is helpful in reducing the loss of volatility of garlic extract. The crushed product were tied in muslin cloth and filtered and the filtrate was collected and centrifuged at 5000 rpm for 15 min. The prepare solution give 100 percent, which was further diluted to required concentrations of 5 and 10 percent. The extracts were heated up to 62 °C for a period of 15 minutes to avoid microbial contamination. Extracts were tested against *Fusarium* sp. Plates containing PDA supplemented with different phyto-extracts, at the respective concentrations and replicated three time were inoculated with 7 day old culture (5 mm dia. disc). Fungal colonies were measured after 7 days of incubation at 25±1 °C.

Evaluations of different phyto extracts were studied *in vitro* by following "Poison Food Technique" against *Fusarium solani* by Schmitz (1930) [10]. The effect of various plant species *viz.*, onion, garlic, ginger, neem leaves, mint and NSKE were tested to know their inhibitory effect on the growth of *F. solani*. Fresh healthy plant parts (leaves, cloves, bulb and rhizomes of prospective bio-control sources) were collected from fields. Leaves of plants were separately washed two-three times in tap water then in distilled water and allowed to dry at room temperature (28 ± 1 °C) for six hours. Prior to extraction, leaves of each plant (100 g) were crushed separately with 100 ml sterilized distilled water except for cloves of *Allium sativum* which was crushed in acetone which is helpful in reducing the loss of volatility of garlic extract. The crushed product were tied in muslin cloth and filtered and the filtrate were collected and centrifuged at 5000 rpm for 15 min. The prepare solution give 100 percent, which were further diluted to required concentrations of 5 and 10 percent. The extracts were sterilized at 62 °C for a period of 15 minutes to avoid microbial contamination. From the 100 ml PDA mixed with extracts, 20 ml poured aseptically into sterilized Petri plates and three plates per treatment were kept. The PDA Petri plate were inoculated with 5 mm mycelial disc cut from the periphery of the *F. solani* culture were grown on PDA medium in the centre with the help of sterilized cork borer. The Petri plates containing PDA media without extract were inoculated with 5 mm mycelial disc cut from the periphery *F. solani* Culture grown on PDA medium were placed in the centre with the help of sterilized cork borer served as a control. The plates were incubated at 28 ± 1 °C temperature in an incubator for seven days. The percent growth inhibition (PGI) of pathogen in each treatments were calculated by following formula given by Bell *et al.* (1982) [2].

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

Where,

I= Percent growth inhibition, C=Colony diameter (mm²) in control plate, T=Colony diameter (mm²) in treated plate

Table 1: List of different phyto extracts tested against *Fusarium solani* *in vitro*

Treatment No.	Common Name	Botanical name	Plant parts for extract	Concentration
T ₁	NSKE	<i>Azadirachta indica</i>	Seed	5%
T ₂	Onion	<i>Allium cepa</i>	Bulb	10%
T ₃	Garlic	<i>Allium sativum</i>	Cloves	10%
T ₄	Neem	<i>Azadirachta indica</i>	Leaves	5%
T ₅	Ginger	<i>Zingiber officinalis</i>	Rhizome	10%
T ₆	Mint	<i>Mentha arvensis</i>	Leaves	10%
T ₇	Control	-	-	-

Table 2: Percent growth inhibition of *Fusarium solani* by various phyto extracts *in vitro* through poison food technique

Sr. No.	Treatment No.	Common Name	Botanical name	Plant parts for extract	Conc.	Average colony diameter of pathogen (mm)	Growth inhibition over control (%)
1.	T ₁	NSKE	<i>Azadirachta indica</i>	Seed	5%	46.62 (52.83)	41.30
2.	T ₂	Onion	<i>Allium cepa</i>	Bulb	10%	48.23 (55.63)	38.18
3.	T ₃	Garlic	<i>Allium sativum</i>	Cloves	10%	62.27 (78.33)	12.96
4.	T ₄	Neem	<i>Azadirachta indica</i>	Leaves	5%	45.94 (51.63)	42.62
5.	T ₅	Ginger	<i>Zingiber officinalis</i>	Rhizome	10%	58.71 (73.00)	18.89
6.	T ₆	Mint	<i>Mentha arvensis</i>	Leaves	10%	48.64 (56.33)	37.40
7.	T ₇	Control	-	-	-	71.56 (90.00)	0.00
	S.Em±					0.68	
	CD at 5%					2.05	
	CV%					2.15	

*Figures in parenthesis are original values while outside are arc sign transformed values



Fig 1: Percent growth inhibition of *Fusarium solani* by various phyto extracts *in vitro*

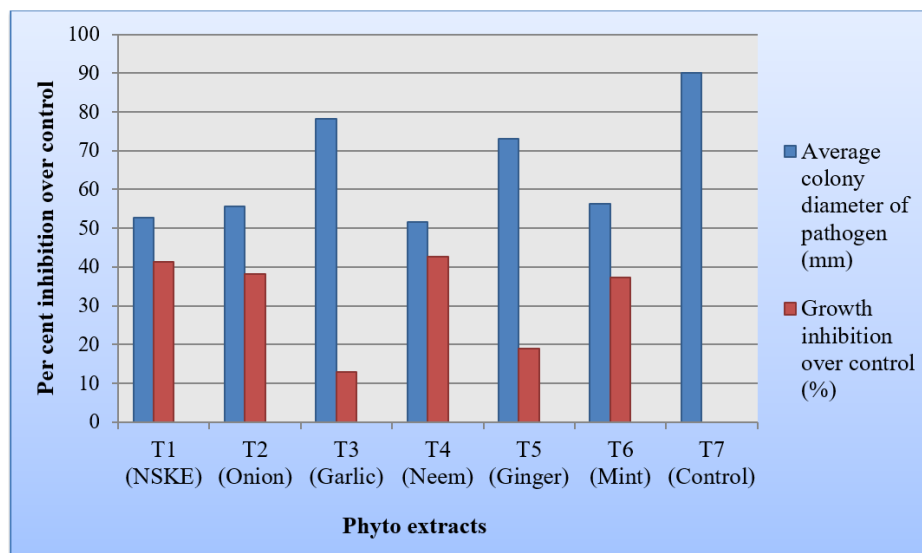


Fig 2: Growth inhibition of *F. solani* by different phyto extracts *in vitro*

From this experiment, it was very clear that extract of onion (*Allium cepa*), garlic (*Allium sativum*), neem (*Azadirachta indica*), ginger (*Zingiber officinalis*), mint (*Mentha arvensis*) and NSKE may have some strong toxic principle present which were directly affect the growth of the *Fusarium solani*. Similar results were recorded by Mamatha and Ravishankar (2004) [8] reported that neem extract was effective in growth inhibition of *F. solani*. Joseph *et al.* (2008) [4] and Obongoya *et al.* (2010) [9] found that neem extracts (*A. indica*) was found most effective in growth inhibition of *F. solani*. According to Khanzada *et al.* (2016) [5] higher dose of *A. indica* extracts

showed maximum inhibitory effect against *F. solani*. Ghante *et al.* (2019) [3] concluded that average mycelial growth inhibition was significantly highest in *A. indica*.

Summery

The phyto extracts of commonly available six different sterilized plants were tested in laboratory condition by poison food technique against *F. solani*. The neem leaf extracts 5% (*Azadirachta indica* L.) (42.62%) was proved significantly superior and at par with NSKE 5% (*A. indica* L.) (41.30%) to inhibit mycelial growth of the pathogen. The next best was

onion (*Allium cepa* L.) followed by mint (*Mentha arvensis*), ginger (*Zingiber officinalis*) and garlic (*Allium sativum*). Garlic (*Allium sativum*) was found least effective to control the pathogen.

Conclusions

It was concluded that botanicals neem leaf extract and NSKE effectively control the growth of the pathogen *in vitro* condition.

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