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In-vitro Efficacy of Plant-Extract against Sudden Death Syndrome (wilt) of Soybean caused by *Fusarium oxysporum* f. sp. *virguliforme*

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A B S T R A C T

Sudden death syndrome (wilt) of soybean caused by *Fusarium oxysporum* f. sp. *virguliforme*. Therefore, the eco-friendly and environmentally safe management of wilt disease with the use of plant extracts is necessary. The aqueous extracts of the eight phyto-extracts evaluated *in vitro* @ 10 per cent concentration were found antifungal to *Fusarium oxysporum* f. sp. *virguliforme*. However, the highest average mycelial growth inhibition recorded in Garlic clove extract *i.e.* 74.81 per cent and the Drumstick seed extract was found comparatively less effective with minimum mycelial inhibition *i.e.* 38.14 per cent, as compare to other plant extract.

Introduction

Soybean [*Glycine max* (L.) Merrill] is a native of northern China and is the most important legume crop in the world. Soybean is called 'Golden bean'. Soybean plants like many others legumes are capable of fixing and utilizing atmospheric nitrogen through symbiotic relationship with *Rhizobium* bacterium at the root of the crops.

The crop thus, improves soil fertility and economizes crop production not only for themselves but also for the next crop grown in rotation especially, cereal crops (Nassiuma and Wasike, 2002)[5]. It has medicinal importance as soya based food helps to control diabetics and melts all kinds of stones in the urinary bladder.

Seeds were primarily used as pulses by the local population and the green and dried vegetative part were used as foliage for cattle. In addition, 100 g soybean contains 240 mg calcium, 690 mg phosphorous, 11.50 mg iron, 432 calories, 10.50 g fats and 426 mg vitamins A, B and D (Nagraj, 1995)[4]. The major soybean growing countries in the world are United States of America, Brazil, Argentina, China, India and European

community (Saxena, 1976)[8].Soybean crop can be attacked by more than 100 pathogens (Sinclair and Schurtleff, 1975)[9]. About 35 pathogens were reported to infect soybean in India (Gupta *et al.,* 2001)[3]. Fungi, nematodes, viruses, bacteria, and phytoplasmas are known to cause diseases of soybean. The soybean crop is presently suffered due to one of the important disease known as sudden death syndrome. The sudden death syndrome disease is called as wilt of soybean. The soybean wilt is caused by *Fusarium oxysporum* f. sp. *virguliforme* (Aoki 2003)[1]. *Fusarium* genus is a soil borne fungus that causes wilt of many crops. In many cases the fungus causing wilt in a particular crop is specific to that crop. In case of soybean, sudden death syndrome caused by the soil borne pathogen *F. solani* f. sp. *glycines* formerly called *Fusarium virguliforme* sp. in recent days which was first observed in Arkansas during 1971 (Roy *et al.,* 1997)[7]. It can cause great damage, as it may reduce the average yield of soybean by up to 59 per cent (Sinclair and Backman 1989)[10].

Materials and Methods

Plant based pesticides which are relatively economical, safe and non- hazardous can be successfully used against the plant pathogenic fungi. The following phyto-extracts / botanicals were selected to know their efficacy in inhibition of *Fusarium oxysporum* f. sp. *virguliforme* (as virulent one). Antifungal activity of different plant extracts were studied under *in-vitro* condition. The nine medicinal plant species *viz.*, Neem, Tulsi, *Parthenium*, Ghaneri, Garlic, *Gliricidia*, Onion and Drumstick were used to study antifungal activity.

Preparation of phyto-extracts

Fresh healthy plant parts (leaves/cloves/bulbs/rhizomes) collected from fields were washed with distilled water and air-dried. Each plant extract was crushed in 100 ml of acetone (Garlic and Onion crushed in 100 ml of distilled water volume by volume. The extract was filtered through double layered, muslin cloth and further filtrated through Whatsman No.1 filter paper using funnel and volumetric flasks (100 ml cap.).

To study the antifungal mechanism of plant extract, the poisoned food technique was used (Nene and Thapliyal, 1982)[6]. An appropriate quantity of each plant extract (100 %) was separately mixed thoroughly with autoclaved and cooled (400C) PDA medium in conical flasks (250 ml cap.) to obtained desired concentrations of 10 % (10 ml solution mixed with 90 ml molten PDA media). The PDA medium amended separately with plant extracts was then poured (10 ml/plate) into sterile glass petri plates (90 mm dia.) and allowed to solidify at room temperature.

After solidification of PDA, all the treatment and control plates were aseptically inoculated by placing in the center a 5 mm mycelial disc obtained from a week old actively growing pure culture of *F. oxysporum*. Plates containing plain PDA without any botanical extract served as untreated control. For each test botanical extract, three plates / treatment were maintained. All these plates were then incubated at 27 ± 20C temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test fungus.

All these plant extracts were evaluated @ 10 % and observations on radial mycelial growth of the test pathogen were recorded at 24 hrs. interval and continued till growth of test pathogen in untreated control plate was fully covered. Per cent inhibition of test pathogen was also calculated by applying the formula given by Vincent, (1927)[13].

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

Where,

C= Growth of the test fungus in (mm) untreated control plates.

T= Growth of the test pathogen in (mm) treated plates.

Results and Discussion

The antifungal activities of eight phyto-extracts were assessed at 10% concentration in the laboratory for evaluation of their efficacy against *Fusarium oxysporum* f. sp. *virguliforme* by using poisoned food technique.

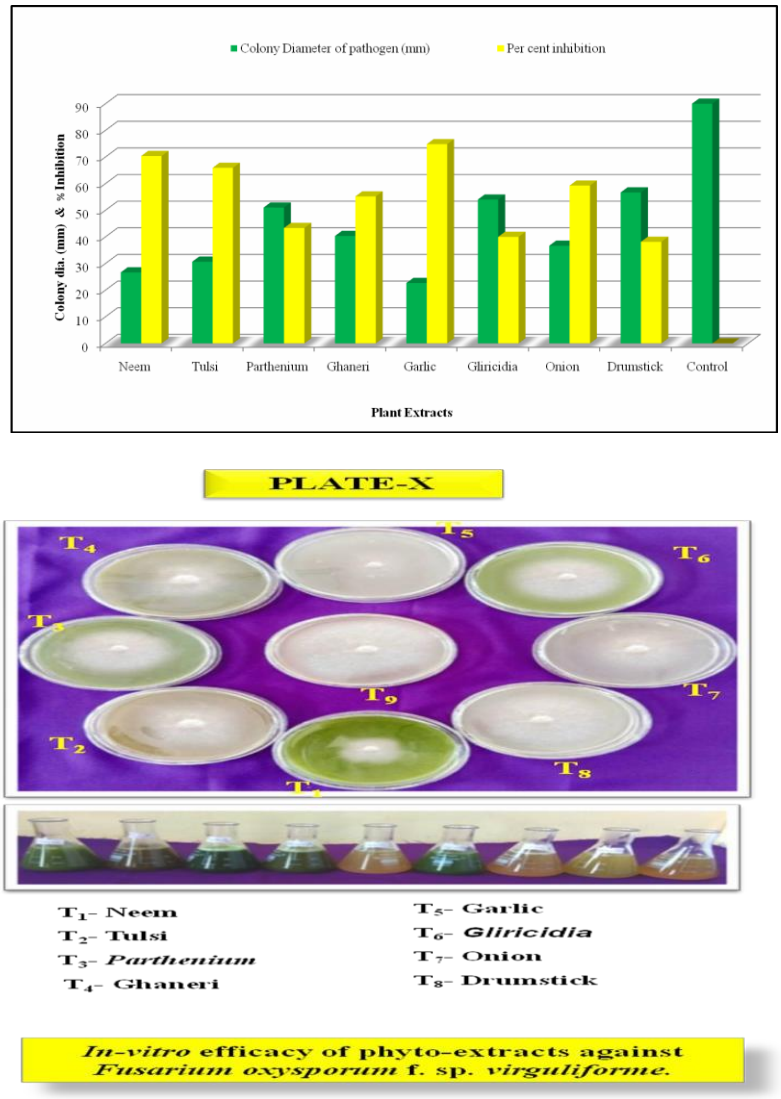
Phyto-extract of eight botanicals were evaluated *in-vitro* (each @ 10 % concentration) against test pathogen and the results obtained on its mycelial growth and inhibition are presented in the Table 1 and depicted in the PLATE X, Fig. 1. Results revealed that all the eight botanical extracts tested were fungaistatic cum antifungal to *Fusarium oxysporum* f. sp. *virguliforme* which were significantly reduced mycelial growth and increased inhibition over untreated control (PLATE X and Fig. 1).

Table.1 *In vitro* evaluation of different plant extracts against *Fusarium oxysporum* f. sp. *Virguliforme*

Tr. No.	Treatments		Colony Diameter of pathogen *(mm)	Per cent inhibition
	Botanical name	Common name		
T1	*Azadirachta indica*	Neem	26.66	**70.36 (57.01)**
T2	*Oscimum sanctum*	Tulsi	30.66	**65.92 (54.28)**
T3	*Parthenium hysterophorus*	*Parthenium*	51.00	**43.32 (41.16)**
T4	*Lantana camera*	Ghaneri	40.33	**55.18 (47.97)**
T5	*Allium sativum*	Garlic	22.66	**74.81 (59.87)**
T6	*Gliricidia sepium*	*Gliricidia*	54.00	**39.99 (39.22)**
T7	*Allium cepa*	Onion	36.66	**59.25 (50.33)**
T8	*Moringa oleifera*	Drumstick	56.66	**38.14 (38.13)**
T9	Control	-	90.00	**00.00 (00.00)**
	SE±		**1.19**	**1.25**
	CD at 1%		**3.56**	**3.75**

*Mean of three replications, Figures in parenthesis are arcsine transformed values

Fig.1 *In vitro* efficacy of different phyto- extracts against *Fusarium oxysporum* f. sp. *virguliforme*



Mycelial growth

At 10 per cent concentration, radial mycelial growth of test pathogen was ranged from 22.66 mm (Garlic clove extract) to 56.66 mm (Drumstick seed extract) as against 90 mm in untreated control. However, significantly least mycelial growth was recorded with 22.66 mm (Garlic clove extract), 26.66 mm (Neem leaf extract). The next best botanical found was 30.66 mm (Tulsi leaf extract). It was followed by botanicals *viz.*, 36.66 mm (Onion bulb extract), 40.33 mm (Ghaneri leaf extract), 51.00 mm (*Parthenium* leaf extract) and 54.00 mm (*Gliricidia* leaf extract). Drumstick seed extract recorded comparatively less effective with maximum mycelial growth of 56.66 mm and still significantly superior over untreated control (90 mm)

Mycelial growth inhibition

Results obtained on mycelial growth inhibition of the test pathogen with the botanicals tested at 10 per cent concentrations are presented in the Table 1, depicted in the

PLATE X and Fig. 1. Result revealed that all the tested botanicals (Plant extract) @ each (10%) concentration significantly superior and also significantly inhibited mycelial growth of the test pathogen over untreated control.

At 10 per cent concentration, mycelial growth inhibition was ranged from 38.14 per cent (Drumstick seed extract) to 74.81 per cent (Garlic clove extract). However, significantly the highest mycelial growth inhibition was recorded 74.81 per cent by Garlic. It was followed by botanicals *viz*., Neem leaf extract (70.36 %), Tulsi leaf extract (65.92 %), Onion bulb extract (59.25 %), Ghaneri leaf extract (55.18 %), *Parthenium* leaf extract (43.32 %). *Gliricidia* leaf extract (39.99 %) and Drumstick seed extract (38.14 %). The lowest mycelial growth inhibition was recorded with Drumstick seed extract (38.14 %) which was at par with *Gliricidia* leaf extract (39.99 %), still these two plant extracts significantly superior over the control.

The results of present investigation resembled with the finding of earlier workers *viz.*, Singh *et al.*, (2017)[11] reported that antifungal activity of twelve botanicals including commercial formulations of neem and garlic at 1, 2, 5 and 10 per cent concentrations was tested against *Fusarium oxysporum* under *in vitro* conditions. Such similar findings mentioned by Dwivedi and Dwivedi. (2012)[2], Suman and Biswas (2017)[12].

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