Biological Forum – An International Journal **15(9): 882-886(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Bio-Efficacy of different Botanical extracts against *Fusarium oxysporum* f. sp. *lentis*, causing lentil wilt under *in-vitro* condition

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(Received: 03 July 2023; Revised: 04 August 2023; Accepted: 07 September 2023; Published: 15 September 2023)
(Published by Research Trend)

ABSTRACT: Present investigation was undertaken for bio-efficacy of aqueous botanical extracts of eight different non-host plants *viz.*, *Azadirachta indica* (NSKE), *Azadirachta indica* (Leaves), *Osimum sanctum* (Leaves), *Datura stramonium* (Leaves), *Calotropis procera* (Leaves), *Lantana camara* (Leaves), *Nerium oleander* (Leaves) and *Parthenium histrophorus* (Leaves) at 5, 10 and 20% concentration were evaluated against *Fusarium oxysporum* f. sp. *lentis*, causing lentil wilt through poison food technique on potato dextrose agar medium. The result revealed that Among all eight botanical extracts, extract of *Osimum sanctum* (Leaves) was found most effective in inhibiting mycelial growth (21.48, 30.93 and 45.19 %) of *F. orysporum* f. sp. *lentis* at 5, 10 and 20 percent, respectively followed by *Azadirachta indica* (NSKE) extract (19.26, 28.52 and 41.85%) over control at all the concentrations tested after 168 hrs. of inoculation. At 5 per cent concentration *Calotropis procera* (Leaves) and *Azadirachta indica* (Leaves) were found at par with 10.74% and 12.96% mycelial growth inhibition, respectively, Extract of *Parthenium histrophorus* (Leaves) was found least effective in inhibiting mycelial growth of *Fusariumo xysporum* f. sp. *lentis* over control 4.07, 10.93 and 17.59 at 5, 10 and 20 percent concentration, respectively.

Keywords: Botanical extracts, Neem Seeds Kernel Extract (NSKE), *Fusarium orysporum* f. sp. *lentis*, Poison food technique.

INTRODUCTION

Lentil (*Lens culinaris* Medik*)* is one of the most important grain legume nitrogen fixing crops. According to Ladizinsky (1979) Lentil has been originated in Southern Turkey. Cubero (1984) in a detailed review concluded that the region between Western Turkeyand Kurdis could be its place of origin. It is cultivated in semi- arid regions of the world particularly in the Indian Sub-continent and the dry areas of Middle East (Malik, 2005). It is a short stature; annual, self-pollinate & high value crop which has great significance in cereal based cropping system. It belongs to Family Fabaceae sub family Papilionaceae. Lentil is bushy, autogamous diploid crop (2n=2x=14).

It is generally grown as rainfed crop during *Rabi* season for its lens shaped seed. Lentil seed contain crude protein about 25-27%, carbohydrates 59%, fat 0.5%, minerals 2.1% and significant amount of vitamins (Gowda & Kaul 1982). The leaves, stems & threshed pods of lentil are important for feeding sheep and goats. Lentil is known by different names in various countries such as *lentiUe* (French), *linse* (German), *lenteja* (Spanish) *a das* (Arabic), *mercimek* (Turkish), *hiramame* (Japanese) and *masur* /*masoor* (Hindi) (Ali and Mishra 2000).

The area of the world under lentil production is 2.5 million ha and the contribution of Indian sub-continent (India, Pakistan and Bangladesh) is about 38% (composition and quality). Lentil is largely consumed as dal and use as food and fodder, the leaves, stems & threshed pods of lentil are important for feeding sheep and goats. It has become an important dietary component in the developing countries like Afghanistan, Bangladesh, Egypt, India, Turkey, Syria, Pakistan, Iraq, Nepal, Spain, China, Morocco, Ethiopia, Tunisia, Sudan, Iran, etc. Many of these countries are major producer too. Globally it is cultivated in India, Canada, Australia, Turkey, Syria, Pakistan, Bangladesh, Spain, China, Morocco, Ethiopia, Chile, Argentina, USA, Oceania etc. In India, lentil is mostly grown in northern plains, central and eastern parts of India. The total area under lentil in India was 14.94 Lakh ha with a total production of 15.06 Lakh tonnes at 1008 kg/ha productivity. In Rajasthan, lentil is grown in Bundi, Kota, Pratapgarh, Bhilwara, Jhalawar and Bharatpur districts covering the total area of 0.31 Lakh hectare, producing 0.43 Lakh tonnes with productivity of 1387 kg/ha, during 2017-18 (Anonymous, 2019).

Yield of lentil remains low due to many biotic and abiotic stresses. A major limiting factor in profitable cultivation of lentil is the attack of several diseases mainly caused by fungi, which cause heavy loss of the crop at all the stages of growth right from sowing to harvest and in storage. Biotic stresses such as fusarium wilt (*Fusarium oxysporum* f. sp. *lentis*), ascochyta

blight (*Ascochyta lentis*), stemphylium blight (*Stemphylium botryosum*), anthracnose (*Colletotrichum truncatum*), root rot (*Rhizoctonia solani*), rust (*Uromyces viciae-fabae*), white mold (*Sclerotinia sclerotiorum*) and collar rot (*Sclerotiun rolfsii*), (Kumar *et al.,* 2013; Sharpe *et al.,* 2013) cause severe yield loss. Productivity of lentil is reduced due to different diseases infecting leaves, stems, roots and pods. It also reduces marketability due to discoloration of seeds.

Among diseases, fusarium wilt caused by *Fusarium oxysporum* f. sp. *lentis* (*Fol*) is one of the major diseases affecting lentil yield all over the world (Bayaa *et al.,* 1998; Khare, 1981). Lentil wilt was first reported from Hungary (Fleischmann, 1937) for the first time, and later on from many countries including India (Padwick, 1941), USA (Wilson and Brandsberg, 1965), USSR (Kotava *et al.,* 1965), Syria (Bayya *et al.,* 1986) and Turkey (Bayya *et al.,* 1998). Globally Lentil wilt is considered as the most harmful soil borne disease of lentil (Khare, 1981; Bayya *et al.,* 1998). *Fusarium oxysporum* f. sp. *lentis* affect lentil at every growth stage like seed, seedling, flowering and at crop maturity in stem and root which causes seed rot, stem rots, damping off, wilt and root (Khare *et al.,* 1979; Vasudeva and Srinivasan 1952). Warm and dry conditions are most ideal condition for proliferation of disease (Bayaa and Erskine 1990).

In India, fusarium wilt is major factor limiting lentil production in the states of Uttar Pradesh, Madhya Pradesh, Himachal Pradesh, Bihar, West Bengal, Assam, Rajasthan, Haryana and Punjab (Agrawal *et al*., 1993; Chaudhary *et al.,* 2009; 2010). The incidence of this disease has been reported at seedling, flowering and pod stages at 25°C temperature or above (Kannaiyan and Nene 1976). Lentil wilt is a serious disease caused by *Fusarium oxysporum* f. sp.*lentis* (*Fol*) and plays major role in reducing lentil yield in India and world (Hamdi and Hassanein 1996). Fusarium wilt causes yield losses up to 50% in India. The disease may appear in early stage of crop growth (seedling) as well as the reproductive stage (adult stage) (Khare, 1981, Stoilova and Chavdarov 2006). Wilt pathogen survives in soil as chlamydospores that can remain viable for several years (Erskine and Bayaa 1996) and is capable of colonizing residues and roots of most crops grown in rotation with lentil. The incidence of wilt disease is increasing, causing substantial lentil yield losses. Many attempts have been made to control this disease using chemical, biological, varietal and cultural methods (Ram and Pandey 2011; Sinha and Sinha 2004; Khan and Mehnaz 2003; Srivastava *et al*., 2000). However, due to its soil borne nature and long survival of pathogen through chlamydospores, it is difficult to manage through conventional method. The application of fungicides although effective, but is un-economical and hazardous to our environment. They not only affect associated beneficial microbiota in soil but also are main source contributing towards environmental pollution. As such, use of alternative methods like eco-friendly and economical management through botanical extracts seems to be more appropriate to manage such soil borne diseases. This disease is more problematic for lentil farmer in the *Hadoti* region of Rajasthan. In view of seriousness of the disease it was considered worthwhile to undertake research with following aspects to device suitable approaches for eco-friendly management of lentil wilt disease through non-host botanical extracts under *in-vitro* condition.

MATERIALS AND METHODS

Present investigation was carried out in the laboratory of Department of Plant Pathology, College of Agriculture, Ummedganj-Kota during my master degree programme.

Collection, isolation, pathogenicity and identification of *Fusarium oxysporum* f. sp. *lentis*: Infected plants which showing typical wilting symptoms were collected during the month of January-February, 2019 from the lentil fields of AICRP MULLaRP, ARS, Ummedganj (Kota). Samples were bringing to laboratory for further studies. Isolation of fungus was carried through standard tissue isolation through infected plant's roots and stems and the pure culture of fungus was obtained by further growing culture and following hyphal tip culture under aseptic conditions was maintained on PDA slants at 4±1°C for further studies. Pathogenicity was proved through soil inoculation. The colonies appeared as pure white mycelial growth were identified by observing the colony against light with the naked eyes and later confirmed sporulation with the help of microscope. On the basis of these characters the pathogen was identified as *Fusarium oxysporum* f. sp. *lentis.* Further, identification of pathogen was confirmed and it is identified by Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi as *Fusarium oxysporum* f. sp. *lentis*.

Bio-efficacy of botanical extracts against the pathogen, *in-vitro*: Anti-fungal activity of various Eight botanical extracts *viz.*, *Azadirachta indica* (NSKE), *Azadirachta indica* (Leaves), *Osimum sanctum* (Leaves), *Datura stramonium* (Leaves), *Calotropis procera* (Leaves), *Lantana camara* (Leaves), *Nerium oleander* (Leaves) and *Parthenium histrophorus* (Leaves) were evaluated *in-vitro* at three concentrations *viz.*, 5, 10 and 20% against *Fusarium oxysporum* f.sp. *lentis*, by poisoned food technique as suggested by Nene and Thapliyal (2018).

Preparation of cold aqueous botanical extracts: Fresh sample of each above mention test plants were collected and washed first in tap water and then in distilled water. 100 g of fresh samples were crushed in a surface sterilized Pestle and mortar or mixer cum juicer by adding 100 ml sterile distilled water (1:1 w/v). The extract was filtered through double layer muslin cloth followed by Whatman's No. 1 filter paper and filtrate was considered as standard extract (100%) & used as stock solution. To study the anti-fungal activity of plant extract, poisoned food technique was followed.

Five, ten and fifteen ml of stock solution was mixed with 95, 90 and 80 ml of sterilized molten PDA medium respectively, as to get 5, 10 and 20 % concentrations. The medium was thoroughly shaken for uniform mixing of the extract after adding the

botanicals, to avoid the bacterial contamination a little amount of streptomycin antibiotic was added at the time of pouring media.

Near about 25 ml of medium was poured into each of 90 mm sterilized petri plates. Each plate was inoculated with 6 mm mycelial disc taken from the periphery of fresh fungal culture. The disc was placed upside down in the center of petri plate, so that the mycelium was in direct contact with the medium poisoned with requisite plant extracts at required concentration.

Three replications were maintained for each treatment and incubated at 28±1°C till growth of colony touched the periphery in the control plate. Suitable control plates were maintained where in culture discs were inoculated into the center of potato dextrose agar plates without plant extracts. Mean colony diameter in each case was recorded by taking the diameter of the colony in two directions. Radial growth of the fungus was measured and percent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947). The data were analysed statistically.

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

Where, I = percent inhibition, C = growth in control, T = growth in treatment.

RESULTS AND DISCUSSIONS

***In-vitro* effect of botanical extracts on mycelial growth inhibition of *F. oxysporum* f. sp. *lentis*, by poisoned food technique at different concentrations.**

Eight botanical extracts *viz.*, *Azadirachta indica* (NSKE), *Azadirachta indica* (Leaves), *Osimum sanctum* (Leaves), *Datura stramonium* (Leaves), *Calotropis procera* (Leaves), *Lantana camara* (Leaves), *Nerium oleander* (Leaves) and *Parthenium histrophorus* (Leaves) at 5, 10 and 20% concentration were evaluated against *F. orysporum* f. sp. *lentis* by poison food technique on potato dextrose agar medium. Data on the effect of three concentrations of eight botanical extracts on the radial growth of *F. orysporum* f. sp. *lentis*have been presented in (Table 1, Fig. 1 (a&b) and Plates 1).

Data presented in Table 1 clearly indicate that, none of the botanical extracts could completely inhibited the growth of *F. orysporum* f. sp. *lentis* even at 20 percent concentration. Among eight botanical extracts, extract of *Osimum sanctum* (Leaves) was found most effective in inhibiting mycelial growth (21.48, 30.93 and 45.19 %) of *F. orysporum* f. sp. *lentis* at 5, 10 and 20 percent, respectively followed by *Azadirachta indica* (NSKE) extract (19.26, 28.52 and 41.85%) over control. Extract of *Parthenium histrophorus* (Leaves) was found least effective in inhibiting mycelial growth of *F. orysporum* f. sp. *lentis* over control 4.07, 10.93 and 17.59 at 5, 10 and 20 percent concentration, respectively.

All the concentrations (5, 10 and 20%) of *Osimum sanctum* (Leaves) extract were found significantly superior over other treatments. At 5 per cent concentration *Calotropis procera* (Leaves) and *Azadirachta indica* (Leaves) were found at par with 10.74% and 12.96% mycelial growth inhibition, respectively. This differential anti fungitoxic activity of different extracts may be due to variation in composition of antifungal compounds in different plants. Present finding agrees with Kanherkar *et al*. (2007) who reported that the leaf extracts of *Azadiracta indica*, *Parthenium hysterophorus*, *Eucalyptus globus* and *Lantana camara* were inhibitory to the growth of *F. oxysporum* and highest inhibition was recorded in *E. globus*. Prakasham *et al.* (2001) also reported that the extracts of Parthenium, Eucalyptus, Neem and Calotropis were responsible for complete inhibition of spore germination of spore germination in 5% and 10% concentrations against *F. solani* f.sp. *radicola*. Bansal and Gupta (2000) also reported *A. indica* was highly toxic to *F. oxysporum* with complete inhibition of growth and sporulation for 100 per cent concentration.

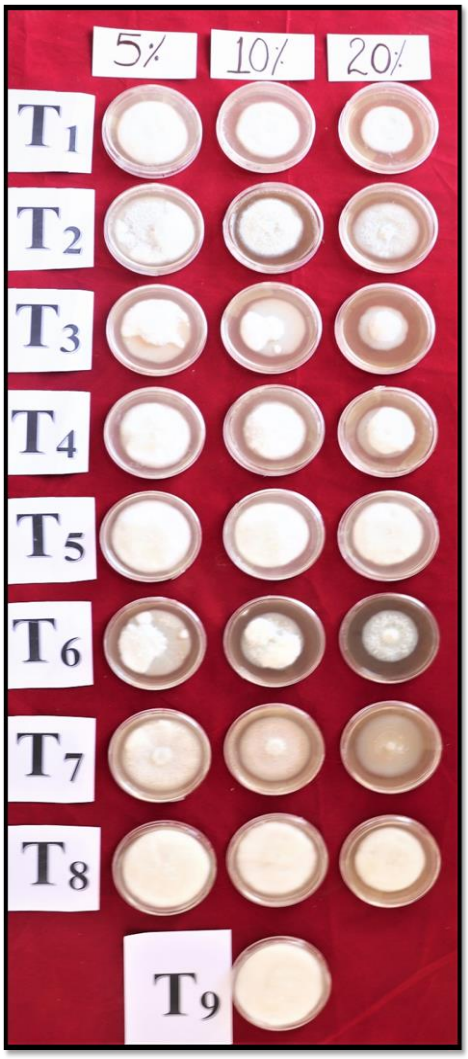

Plate 1. *In-vitro* efficacy of botanical extracts on mycelial growth inhibition of *Fusarium oxysporum* f. sp. *lentis* by poisoned food technique at different concentrations.

Table 1: *In-vitro* efficacy of botanical extracts on mycelial growth inhibition of *Fusarium oxysporum* f. sp. *lentis* by poisoned food technique at different concentrations.

Sr. No.	Botanicals with plant parts used	Mycelial growth of *Fusarium oxysporum* f. sp. *lentis* * (mm)				Percent mycelial growth inhibition of *Fusarium oxysporum* f. sp. *lentis* *			
		5%	10%	20%	Mean	5%	10%	20%	Mean
1.	T_1: *Azadirachta indica* (NSKE)#	72.67	64.33	52.33	63.11	19.26 (26.03)**	28.52 (32.27)	41.85 (40.31)	29.88 (32.87)
2.	T_2: *Azadirachta indica* (Leaves)	78.33	70.67	64.67	71.22	12.96 (21.09)	21.48 (27.61)	28.15 (32.04)	20.86 (26.91)
3.	T_3: *Osimum sanctum* (Leaves)	70.67	62.17	49.33	60.72	21.48 (27.61)	30.93 (33.79)	45.19 (42.24)	32.53 (34.54)
4.	T_4: *Datura stramonium* (Leaves)	75.00	66.33	56.67	66.00	16.67 (24.09)	26.30 (30.85)	37.04 (37.48)	26.67 (30.81)
5.	T_5: *Calotropis procera* (Leaves)	80.33	76.67	70.67	75.89	10.74 (19.09)	14.81 (22.63)	21.48 (27.60)	15.68 (23.11)
6.	T_6: *Lantana camara* (Leaves)	77.17	67.17	58.17	67.50	14.26 (22.18)	25.37 (30.24)	35.37 (36.49)	25.00 (29.64)
7.	T_7: *Nerium oleander* (Leaves)	82.17	78.33	72.33	77.61	8.70 (17.15)	12.96 (21.10)	19.63 (26.30)	13.77 (21.51)
8.	T_8: *Parthenium histrophorus* (Leaves)	86.33	80.17	74.17	80.22	4.07 (11.53)	10.93 (19.28)	17.59 (24.79)	10.86 (18.53)
9.	T_9: **Control**	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Concentrations Mean		79.19	72.87	65.37	72.48	12.02 (18.75)	19.03 (24.20)	27.37 (29.69)	19.47 (24.21)
		S Em±	**CD at 5%**	**CV (%)**		**S Em±**	**CD at 5%**	**CV (%)**	
Fungicides		0.31	0.89			0.35 (0.21)	0.99 (0.59)		
Concentrations		0.18	0.51			0.20 (0.12)	0.57 (0.34)		
F × C		0.54	1.54	1.30		0.60 (0.50)	1.71 (1.41)	5.37 (3.55)	

NSKE = Neem Seed Kernel Extract
*Average of three replications; **Figures in parentheses are Arc sine transformed values.

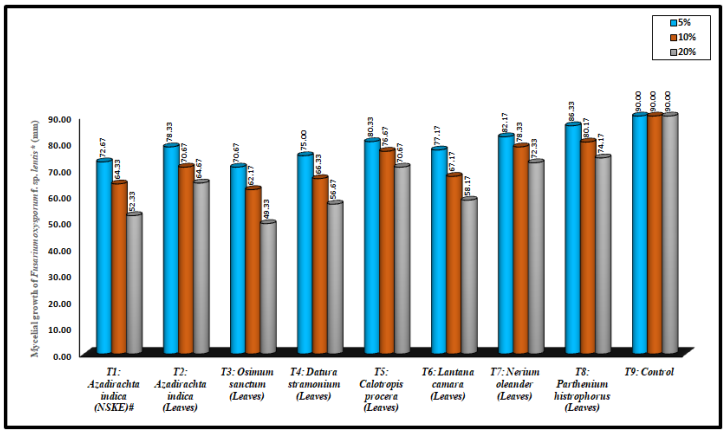

Fig. 1(a) *In-vitro* efficacy of botanical extracts on mycelial growth of *Fusarium oxysporum* f. sp. *lentis* by poisoned food technique at different concentrations.

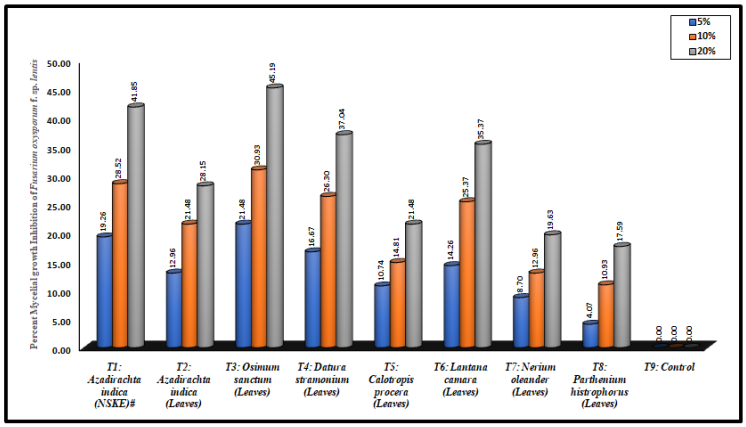

Fig. 1(b) *In-vitro* efficacy of botanical extracts on percent mycelial growth inhibition of *Fusarium oxysporum* f. sp. *lentis* by poisoned food technique at different concentrations.

CONCLUSIONS

Botanical's control is very important aspects to minimize cost of cultivation and to avoid environment pollution and health hazards. The result revealed that Among all eight botanical extracts, extract of *Osimum sanctum* (Leaves) was found most effective in inhibiting mycelial growth (21.48, 30.93 and 45.19 %) of *F. orysporum* f. sp. *lentis* at 5, 10 and 20 percent, respectively followed by *Azadirachta indica* (NSKE) extract (19.26, 28.52 and 41.85%) over control at all the concentrations tested after 168 hrs. The results however need field evaluation before these recommended to farmers.

Acknowledgement. The authors thank Agriculture University, Kota for financial support and Dr. C. B. Meena, (Major Guide) for their moral support and Department of Plant Pathology, College of Agriculture, Ummedganj-Kota for all facilities.
Conflict of Interest. None.

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How to cite this article: Karan Singh, Meenu Kumari Meena, C.B. Meena, Chirag Gautam and Hemant Gurjar (2023). Bio-Efficacy of different Botanical extracts against *Fusarium oxysporum* f. sp. *lentis*, causing lentil wilt under *in-vitro* condition.. *Biological Forum – An International Journal*, *15*(9): 882-886.