ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2015; 1(13): 09-13
www.allresearchjournal.com
Received: 08-10-2015
Accepted: 10-11-2015

Jyoti Srivastava
Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A central) University, Lucknow-2260225 U.P, India.

SK Dwivedi
Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A central) University, Lucknow-2260225 U.P, India.

Correspondence
Jyoti Srivastava
Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A central) University, Lucknow-2260225 U.P, India.

Assessment of antifungal potentials of some plant extracts against chickpea wilt

Jyoti Srivastava, SK Dwivedi

Abstract
Chickpea enjoys a major share in the pulse cultivation across the globe because of its multifarious uses and its pivotal utility in human diet. Fusarium wilt caused by *Fusarium oxysporum f.sp. Ciceri* is amongst one of the major constraints limiting its yield. The present study addresses the efficacy of four plant extracts in controlling the growth *Tamarindus* of wilt pathogen. For the present study aqueous and alcoholic extracts of *Callistemon lanceolatus, indicus, Terminalia Arjuna* and *Zizyphus jujuba* were prepared at three different concentrations *viz.* 100, 200 and 500ppm concentrations. Alcoholic extracts of all the tested plants were superior as compared to their water counterparts at all concentrations. At 500ppm alcoholic extract of *Callistemon lanceolatus* was found to be the most effective among the tested plant varieties inhibiting the growth to (97.55%) followed by *Zizyphus jujuba* (70.62%), *Tamarindus indicus* (61.68%) and *Terminalia arjuna* (58.89%).

Keywords: Chickpea, wilt, plant extracts.

1. Introduction
Grain legumes play a pivotal role in improving the livelihood, nutritional security of farmers and populations in less developed countries as well as in sustainable agriculture of the arid and semi-arid regions worldwide. Chickpea (*Cicer arietinum* L. diploid, 2n=16) is considered to be one of the founder crops of modern agriculture [1]. It is an important source of human food and animal feed and a significant factor in improving soil fertility.
Of the many biotic stresses limiting its production in the Mediterranean basin and Indian subcontinent Fusarium wilt caused by *Fusarium Oxysporum* f. sp. *ciceri* is the most notable [2]. Heavy annual losses are encountered due to Fusarium wilt epidemic which may reach 100% under conditions favorable for disease [3-4]. Conventional approach of disease management through chemical pesticides is under public scrutiny due to the potential harmful effects on environment, their undesirable effects on non-target organism and possible carcinogenicity [5]. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market [6]. Other practices like crop rotation soil solarization, pathogen free seed have been employed but with limited success.
Hence persistent effort to seek better alternative is inevitable. An interesting vista is the use of natural plant products in controlling the fungal disease due to their being ecofriendly [7] and positive role in sustainable agriculture. Phytofungicides could be prepared or formulated from the leaves, seeds, stem bark or roots of plants and could be applied in the form of extract, powders and cakes or as plant exudates [8].Vast fields in developing countries are blessed with abundant plants with fungicidal potential with preparation and application attracting lower capital investment than synthetic fungicides [9]. Plant extract have been tested against *Fusarium oxysporum* for their inhibitory effect and their control efficacy under greenhouse conditions [10-12] .The present study was undertaken to evaluate the potential of some local plant leaves extracts in reducing the population of *Fusarium oxysporum* f.sp. *ciceri*.

2. Material Method
2.1 Isolation and identification of the test fungal strain
The pathogen *Fusarium oxysporum* f. sp. *ciceri* (FOC) used in the present study was isolated from roots of wilt infected chickpea plants collected from the farm fields of Kanpur and

Unnao district. Roots from diseased plant specimens were sliced into 2 to 3 bits and placed in mercuric chloride (0.01%) solution for 3 minutes and rinsed with sterile distilled water. The pieces were then picked using a pair of sterile forceps, blotted dry and placed in Potato Dextrose Agar (PDA) plates and incubated for 7 days at room temperature (27 ± 2 °C). The isolated fungi was identified on the basis of colour, morphological characters as well as sporulating structure and conidia under microscope and confirmed by available literature [13].

2.2 Source of plant material

Callistemon lanceolatus, Tamarindus indicus, Terminalia arjuna and *Zizyphus jujuba* were collected from nearby areas of Kanpur and Unnao district. Fresh plant material was collected in resealable plastic bags.

2.3 Determination of mycelial inhibition by poisoned food technique

In order to study the effectiveness of some local trees as botanical toxicant , leaves of 4 medicinal plants species viz. *Callistemon lanceolatus, Tamarindus indicus, Terminalia arjuna* and *Zizyphus jujuba* were collected and washed with sterile water. The leaves were air dried and then grinded with the help of pestle and mortar. It was then crushed with equal amount of water (w/w).Centrifuged at 5000rpm for 15 minutes. The filtrate was then taken as 100% stock solution. For the preparation of alcoholic extract alcohol was used instead of water. The extracts were poured in screw cap bottles to avoid contamination. Concentrations of 100, 200 and 500ppm were prepared in PDA medium amended with leaves extract as in food poison technique [14]. The medium in petriplates was then inoculated from 7 day old culture of the pathogen. The Petri dishes containing media devoid of the extracts with same amount of distilled water served as control. Inoculated petriplates were kept at 27 ºC±1in BOD Incubator. Each experiment was then replicated thrice and percentage inhibition was then calculated according to the equation:

$$\% (I) = (dc-dt))/dc \times 100$$

Where I is percentage inhibition, dc is growth of the pathogen in control plate and dt is growth of the pathogen in treatment set plate.

2.4 Statistical analysis

All values were expressed as mean ± SD, n= 3 and the results on the percent reduction of colony growth of the FOC isolates *in vitro* were analyzed by analysis of variance (ANOVA). $P \leq 0.05$ was considered statistically significant.

3. Result

Plant extract obtained from four medicinal plants were evaluated for their potential fungitoxicity. We compared percent inhibition of *Fusarium oxysporum* f .sp. *ciceri* using alcoholic and water extract of the above mentioned four plants at different concentrations as presented in Table 1. One way ANOVA, (followed by Dunnett's multiple comparison test) Test was used to make all comparisons. The assay was performed in triplicate and SD has been shown by error bars. At 100ppm, 200ppm and 500ppm concentrations of different plant extracts, percent inhibition of *Fusarium oxysporum* f .sp. *ciceri* were significant ($P<0.001$). It was observed that increasing the concentration of the plant extract both of water and of alcoholic extract caused an increase in the percent inhibition of the radial growth of the pathogen *Fusarium oxysporum* f.sp. *ciceri* (FOC). However, it was noticeable that *Callistemon lanceolatus* and *Zizyphus jujuba* proved to be more promising fungitoxicant. At 500ppm alcohol extract of *Callistemon lanceolatus* significantly controlled the growth to (97.55%) ($P \leq 0.001$) followed by *Zizyphus jujube (*70.62%) *Tamarindus indicus* (61.68%) and *Terminalia arjuna* (58.79%) while aqueous extract of *Callistemon lanceolatus* at 500ppm controlled the growth upto (92.20%), *Zizyphus jujuba* (57.26%), *Tamarindus indicus* (45.51%). *Terminalia arjuna* was the least effective among all the tested plant varieties and showed only (37.91%) reduction with water extract in the colony growth of the pathogen.

Table 1: Percent inhibition in the radial growth of *Fusarium oxysporum* f. sp. *ciceri* at different concentrations of water and alcohol extracts:

S. No	Name of plants	Concentration in ppm	Water extract		Alcohol extract	
			Radial growth in mm	Percent Inhibition	Radial growth in mm	Percent inhibition
1	*Callistemon lanceolatus*	100	40.43±0.75	40.36±1.11	33.1±1.15	50.69±1.71
		200	29.06±0.90	57.71±1.31	25.26±1.10	63.11±1.60
		500	5.4±0.52	92.20±0.76	1.66±0.6	97.55±0.84
2	*Tamarindus indicus*	100	53.13±1.02	21.62±1.51	47.96±1.26	28.54±1.88
		200	50.1±1.01	27.11±1.47	42.1±0.85	38.54±1.24
		500	37.73±0.64	45.51±0.92	26.13±1.02	61.68±1.50
3	*Terminalia arjuna*	100	55.2±0.64	18.48±0.94	50.4±1.25	24.87±1.86
		200	51.76±0.68	24.69±0.99	46.13±1.10	32.65±1.61
		500	43.00±1.00	37.91±1.44	28.1±1.01	58.79±1.48
4	*Zizyphus jujube*	100	51.06±1.00	24.68±1.48	46.4±0.52	30.88±0.78
		200	44.06±1.10	35.89±1.60	41.13±0.80	39.95±1.17
		500	29.6±1.21	57.26±1.75	20.03±1.05	70.62±1.54
	Control	100	67.8±1.31		Control	67.13±1.80
		200	68.74±1.09			68.5±1.32
		500	69.26±0.64			68.2±0.9

Values shown are the mean ± SD of 3 replicates, significant at $p \leq 0.05$

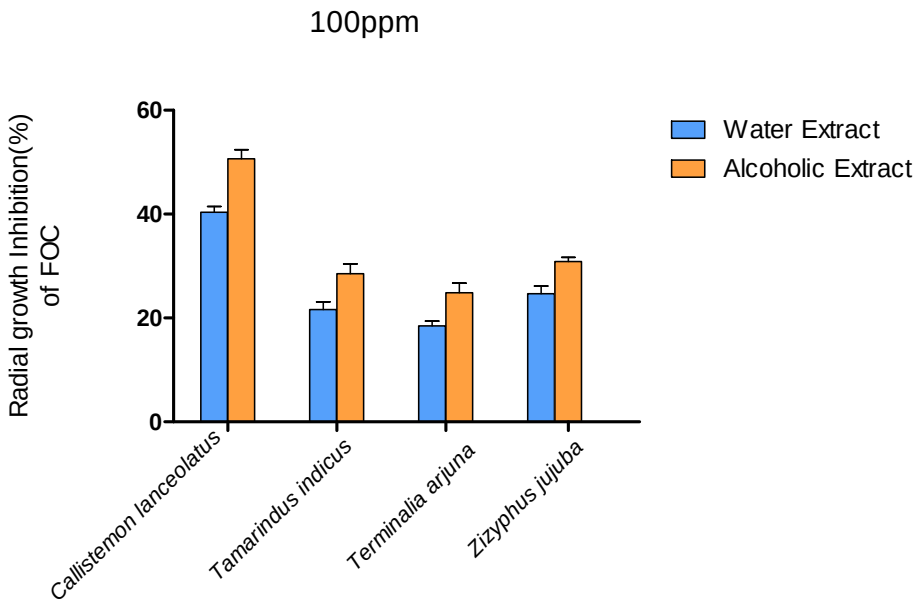

Fig 1: Radial colony growth inhibition (%) of FOC at 100ppm

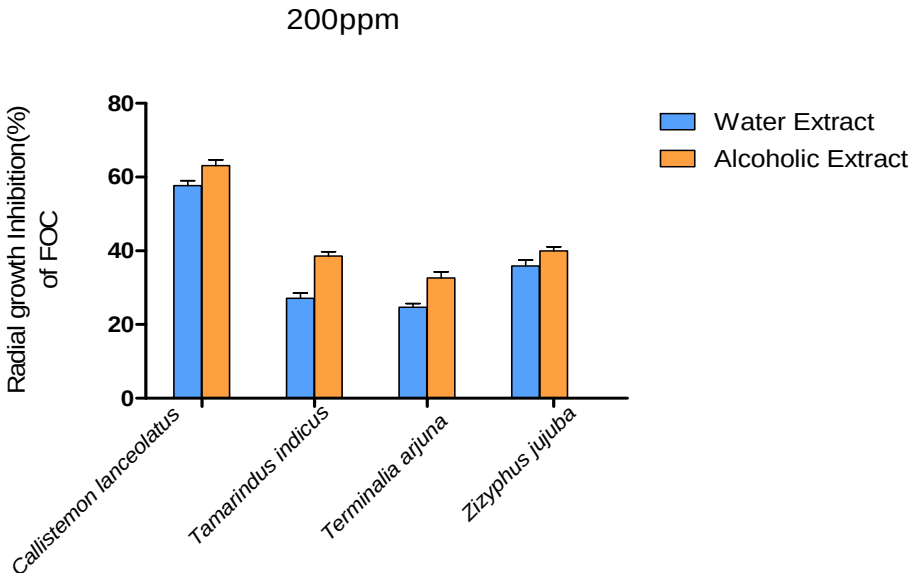

Fig 2: Radial colony growth inhibition (%) of FOC at 200ppm

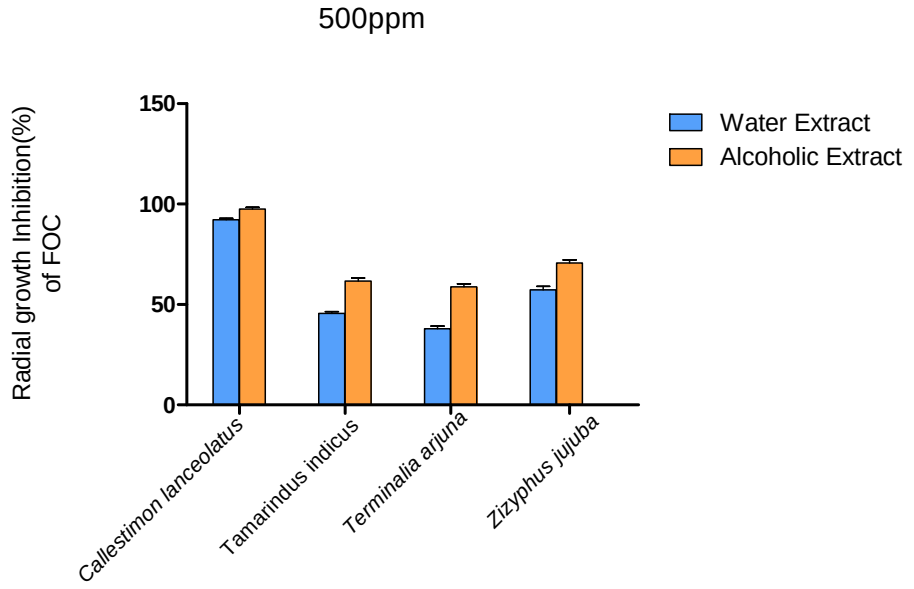

Fig 3: Radial growth inhibition (%) of FOC at 500ppm

4. Discussion

In the recent years, the need to develop fungal disease control measures using phytochemicals as alternative to synthetic chemicals has become a priority of scientists worldwide [15]. Ark and Thompson [16] showed that garlic extracts contain a potent fungicide. They were able to effectively protect peaches against brown rot (*Monilinia fructicola*) with deodorized garlic extract preparations. Singh *et al.* [17] found that essential oils from *Cymbopogon martinii*, *C. oliveri*, and *Trachyspermum ammi* exhibited strong antifungal activity against *Helminthosporium oryzae.* Plant fungicides have been reported to be safe to beneficial organisms such as pollinating insects, earthworms and to humans [18]. Khalid *et al.* [19] reported that their toxic effect is normally of an ephemeral nature disappearing within 14-21days. Antifungal action of plant extracts has great potential as they are easy to prepare and apply. Several authors have confirmed the antifungal properties of several plant parts and phytochemicals [20-22]. The active constituents which are considered responsible for the antifungal properties of various phyto-chemicals are generally low molecular weight phenolics (hydroxybenzoic acid, flavanoids, hydroxycinnamic acid, acetophenone, stilbenes and lignans) as well as oligo or polymeric forms such as hydrolysable and condensed tannins and lignins [23-24]. Antimicrobial properties of numerous plant extracts, polar and non-polar fractions, their pure compounds, and essential oils have been investigated by many researchers against different strains of *Fusarium* [25-29].

5. Conclusion

A large number of earlier workers have reported anti-fungal properties of several plant species. Anjorin [32] reported that combination of two or more plant extracts proved more effective and could reduce the risk of resistance developing by the target fungi. The present study indicates that plant extracts of *Callistemon lanceolatus*, *Tamarindus indicus*, *Terminalia arjuna* and *Zizyphus jujuba* can serve as a cheap, easily available, cost effective, and a holistic option of managing the wilt of chickpea.

6. Acknowledgement

The authors are grateful to HOD environmental science BBAU University for their continued support and guidance. Thanks are also due to Dr. Nisha Rathor (VPCI, University of Delhi) for her indispensable advice and editing of the manuscript.

7. References

1. Zohary D, Hopf M. Domestication of Plants in the Old World: the Origin and Spread of Cultivated Plants in West Asia, Europe, and the Nile Valley, third ed. Oxford University Press, Oxford, UK, 2000.
2. Jalali BL, Chand H. Chickpea wilt. Plant Disease of International Importance. Vol. I. Diseases of Cereals and Pulses. US Singh, AN Mukhopadhayay, J Kumar, and HS Chaube, eds. Prentice Hall, Englewood. Cliffs, NJ. 1992, 429-444.
3. Navas-Cortés JA, Hau B, Jiménez-Díaz RM. Yield loss in chickpea in relation to development of Fusarium wilt epidemics. Phytopathology 2000; 90:1269-1278.
4. Anjaiah V, Cornelis P, Koedam N. Effect of genotype and root colonization in biological control of Fusarium wilts in pigeon pea and chickpea by *pseudomonas aeruginosa* PNA1. Can. J. Microbiol. 2003; 49:85-91.
5. Cook R, Baker KF. The nature and practice of biological control of plant pathogens. The APS. St. Paul. Minnesota.1983; 539.
6. Pal KK, Gardener BS. Biological control of plant pathogens. The Plant Health Instructor. 2006; 10(1094), 1117- 02.
7. Cao KQ, Forerr HR. Current statues and prosperity on biological control of potato late blight *Phytophthora infestans*, Journal of Agricultural Research, Univ. Dubai, 2001; 24(2):51-58.
8. Owino PO, Waudo SW. Medicinal plants of Kenya: Effects of *Meloidogyne incognita* and the growth of okra. Afro-Asian Journal of Nematology. 1992, 64-66.
9. Anjorin ST, Salako EA. The status of pesticidal plants and materials identification in Nigeria. Nigerian Journal of Plant Protection 2009; 23:25-32.
10. Bowers JH, Locke JC. Effect of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of Phytophthora Blight in the greenhouse. Plant Diseases, 2004; 88(1):11-16.
11. Sahayaraj K, Namasivayam SKR, Borgio JAF. Influence of tree plant extracts on *Fusarium oxysporum* f.sp. *ciceri* mycelium growth . Journal of plant Protection Research. 2006; 46(4):335-338.
12. Amadi JE, SO Salami, CS Eze. Antifungal properties and photochemical screening of extracts of African Basil (*Ocimum gratissimum* L.), Agriculture and Biology Journal of North America. 2010; 1(2):163-166.
13. Booth C. The genus, Common Wealth Mycological Institute, Kew Surrey England.1971.
14. Zentymer GA. A laboratory method for testing soil fungicides with *Phytophthora cinnamomi* as a test organism J. phytopathology.1955; 45(3).
15. Reddy CS, Reddy KRN, Prameela M, Mangala UN, Muralidharan K. Identification of antifungal component in clove that inhibits *Aspergillus* spp. Colonizing rice grains. Journal of Mycology and Plant Pathology. 2007; 37(1):87–94.
16. Ark PA, Thompson JP. Control of certain diseases of plants with antibiotics from garlic (*Allium sativum* L.) Plant Dis. Rep. 1959; 43:276-282.
17. Singh AK, Dickshit A, Sharma ML, Dixit SN. Fungitoxic activity of some essential oils. Econ. Bot. 1980; 34:186-190.
18. Rotimi MO, Moens M. The use of leaf extracts of some herbs in the control of *Meloidogyne incognita.* Proceedings of Nigerian Society for Plant Protection. 2003; 21: 34-9.
19. Shahida Khalid, Tahira Ahmad, Shad RA. Use of Allelopathy in Agriculture. Asian Journal of Plant Sciences, 2002; 1:292-297.
20. Giridhar P, Reddy SM, Effect of some plant extracts on citrinin production by *P. citrinum in vitro.* Journal of Indian Botanical Science. 1996; 75:153-154.
21. Benharref A, Jana M. Antimicrobial activities of the leaf extracts of two Moroccan *Cistus* L. species. Journal of Ethnopharmacology. 2006; 104:104-111.
22. Satish S, Mohana DC, Ranhavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology. 2007; 3(1):109-119.

23. Close DC, McArthur C. Rethinking the role of many plant phenolics protection from photo damage. Okios99. 2002:166–172.
24. Okwu DE. Phytochemical and vitamin contents of indigenous spices of South Eastern Nigeria. Journal of Sustainable Agriculture and Environment. 2004; 6:30-37.
25. Gomez-Rodriguez O, Zavaleta-Mejia E, Gonzalez-Hernandez VA, Livera-Munoz M, Cardenaz Soriano E. - Allelopathy and microclimatic modification of intercropping with marigold on tomato early blight disease development. Field crop Research. 2003; 83:27–34.
26. Irum M. Comparison of phytochemical and chemical control *Fusarium oxysporum f.sp. ciceri*. Mycopathology. 2007; 5(2):107-110.
27. Riaz T, Khan SN, Javaid A. – Antifungal activity of plant extracts against *Fusarium oxysporum* the cause of corm rot of *Gladioulus*. Mycopathology. 2008; 6(1,2):13–15.
28. Hassannein NM, Zeid A, Youssef KA, Mahmoud DA. Efficacy of leaf extracts of neem (*Azardirachta indica*) and chinaberry (*Melia azedrach*) against early blight and wilt diseases of Tomato. Australian Journal of Basic and Applied sciences. 2008; 2(3):763-772.
29. Ghorbany M, Jafarpour B, Rastegar MF. Application of some plant products on control of *Fusarium oxysporum f sp. cumini* causing cumin wilt. Journal of plant protection. 2010; 24(1):34–37.
30. Naganawa, R, Iwata N, Ishikawa K, Fukuda H, Fujino T, Suzuki A. Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. Appl. Environ. Microbiol. 1996; 62:4238-4242.
31. Kubo, A, CS Lunde and Kubo I. (Antimicrobial activity of the olive oil flavor compounds. Journal .of Agricultural. Food Chemistry. 1995; 43:1629-1633.
32. Anjorin, ST. Quality Assessment of Botanical pesticides in Nigeria. Proceeding of the 42nd Annual Conference of the Agricultural Society of Nigeria held at Ebonyi State University Abakaliki- Nigeria. 2008; 19th-23rd, November, 130-135.