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RESEARCH ARTICLE

Assessment of antifungal activity of phytoextracts on Fusarium wilt of lentil

■ **Pawan L. Deshmukh and Umesh P. Mogle**

SUMMARY

The cultivation of pulses has been practiced from thousands of years. Ancient civilizations in Mesopotamia grew peas, beans and lentils as far back as 8 000 BC. India is one of the second largest lentil producers in the world after Canada. In South Asian cuisine, split lentils are known as*Dal*,the lentil is a dietary staple throughout regions of India and other developing countries in Asia. Majority of the production of lentil comes from Canada, India and Australia from total world production. These staple crops have been an integral part of human diets for millennia and today are an important crop not only for food security, but also for combating malnutrition, improving human health, alleviating poverty and enhancing agricultural sustainability. However, yield of lentil remains low due to biotic and abiotic stresses. One of the major biotic stress comes from Fusarium wilt (*Fusarium oxysporum* f.sp. lentis) affect lentil and cause severe yield loss. In order to overcome biotic stress caused by Fusarium species eco-friendly biological methods has to be discovered in order to avoid the huge loss due to such biotic stress. This study mainly focused on the development of biological methods to combat against these biotic stresses in environmental friendly manner.

Key Words : Antifungal, *Fusarium oxysporum,* Pulses, Biotic stresses

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MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
Pawan L. Deshmukh, Department of Botany, J. E. S., R. G. Bagdia Arts, S.B. Lakhotia Commerce and R. Benzonji Science College, **Jalna (M.S.) India**
Email : pawandeshmukh5@gmail.com

Address of the Co-authors:
Umesh P. Mogle, Department of Botany, J. E. S., R. G. Bagdia Arts, S.B. Lakhotia Commerce and R. Benzonji Science College, **Jalna (M.S.) India**
Email : upmogle@gmail.com

Lentil is planted as rotational crop for deriving ecological and environment benefits by improving rhizosphere diversity through biological nitrogen fixation increase in fertility of soil, carbon sequestration, and by management of diseases, weeds and insect pests. It is an economical source of proteins, carbohydrates, minerals and fibre for resource poor (Meena *et al.*, 2017). Lentil (*Lens culinaris* sp. culinaris) is an important food legume crop with global cultivated area of lentil is around

4.34 million hectares producing 4.95 million tons of production with an average production of 1140 kg/ha (FAOSTAT,2014). In India lentil was grown in 1.89 mha with production of 1.13 mt with an average production of 598 kg/ha during 2013-14. However, the crop suffers substantial yield losses from various biotic and abiotic stresses. Fungi are major pathogens of lentil and cause Fusarium *wilt*, collar rot, wet root rot, dry root rot, stem rot and damping-off. Among them Fusarium wilt is the most widespread and important disease. It is prevalent in almost every country where lentil is grown. The disease can cause complete failure of the crop, especially in a warm spring and dry hot summer. The fungus is mainly soil-borne but can also be isolated from infected seeds of lentil (Khare,1981; Saxena,1993; Infantino *et al.*, 2006 and Anonymous, 2010). *Fusarium oxysporum* f. sp. Lentis is important and predominant fungus of lentil as compared to other pathogens and reported, wherever lentil is cultivated. Infection starts in the seedling stage and proceeds later stages of growth and development of plants (Mohammadi *et al.*, 2012). Transmission of the fungus occurs primarily through plant debris and soil contamination, where it infects the plant through the root system. There is also evidence of transmission through seeds (Erskine, 1992). Researchers having strong opinions about diversity in virulence of Fusarium isolates while; Belabid *et al.* (2004) and Erskine *et al.* (1990) reported that pathogenic diversity is due to the differences in pathogenic aggressiveness. After infecting host roots, the fungus travels to the cortex and enters the xylem tissues and spreads rapidly up through the vascular system and causes systemic infection in the host tissues. Sometimes directly infect the seeds and causes infestation.

The entry of fungal spores or mycelium takes place by growing healthy plants in contaminated soil by two ways as through direct entry in to the wounds, or the point where lateral roots are formed. The mycelium then enters to the cortex through intracellular path followed by entry in to the xylem vessels through the pits. The microconidia are formed due to confinement of the mycelium branches in to the xylem vessels after that microconidia detaches and carried to upward regions of the vascular system until movement is stopped. After restriction of the movement mycelium germinate and penetrates the adjacent vessels through the walls. Mycelium moves between the vessels through the pits. Due to propagation of the mycelium the plant suffers from water stress due to blockage of vessels, which leads to closure of stomata, wilting and death of leaves and sometimes death of the whole plant. The fungus then spreads through all tissues of the plant and reach the surface where it sporulates in large amount. Spores are then spreads through the vehicles such as wind, water or due to movement of contaminated soil or plant debris. The fungal mycelium and spores resides in the contaminated materials for more than 6 years and cause active infection in suitable environmental conditions. (Singh *et al.,* 2007). Lentil resistant varieties are not immune to the diseases. However, sometimes conditions favour for the spread of 1disease, crop may surrender to the pressure and undergo some sort of damage (Anonymous, 2010 and Chaudhary *et al.*, 2006). *Fusarium oxysporum* maximum damage recorded was (66.3%) as compared to other fungi with lentil. found that the degree of *F. oxysporum* infection ranged from 25-95 per cent depending on the cultivar tested. Wilt incidence during reproductive growth was correlated with yield loss estimates, with a reduction in seed yield per unit change in wilt incidence. Anonymous (2012) reported maximum association of *Fusarium oxysporum* (66.3%) as compared to other fungi with lentil. Keeping in view, the food value and importance of pulse crops in the country; the present research work was planned to find the level of immunity in the available varieties. Disease management is required to ensure the stable lentil production. Application of fungicide is one of the solutions to overcome this problem but field applications are not feasible due to the expense required and technical difficulty in infusing chemicals into the soil. The most sustainable and effective solution to this problem is the development of resistant cultivars (Kraft *et al.,* 1994 and Bayaa *et al.*, 1995). The management of pathogen is difficult, because of its wide host range and ability to grow saprophytically or survive for extended periods in the form of thick walled chlamydospores in absence of a susceptible crop. Excessive use of chemical fungicides may apparently lower the density of pathogens in soil for short period of time but might give rise to mutant strains of pathogens with altered pathogenicity; making the previously resistant varieties of crops susceptible. Use of bio-control methods, crop rotations and genetically modified crops are some of the methods of disease management to curb down extreme use of chemicals on field to control pathogens (Yadav *et al.*, 2017). Here we are going to assess the antifungal activity of biological plant extracts on the growth of *Fusarium oxysporum* species.

MATERIAL AND METHODS

The suspected plants of the lentil for Fusarium wilt are selected from different farms of Jalna district of Maharashtra and *F. oxysporum* was isolated from these plants and cultured on 3 per cent PDA. The pure cultures of the Fusarium species were identified biochemically and physical observation.The selected Fusarium species are then inoculated and maintained in separate plates. To access the antifungal activity of the different plants extracts on the cultured Fusarium species following protocol was followed.

Preparation of plant extracts:

Plant extracts were prepared by washing the plant leaves by sterile distilled water and then dried under normal sunlight. After drying the leaves were grinded by using mortal and pestal and then 3 grams of powder was soaked in 100 ml ethanol for about 4 hours and then filtered through filter paper. Plant extracts then diluted with PDA agar to get final concentration of 10 per cent, 15 per cent, 20 per cent, 25 per cent and autoclaved for 15 minutes. Then plating was performed after mild cooling of autoclaved extracts. Inoculation and incubation of *F. oxysporum* fungus to access antifungal activity. The prepared Petri plates are then inoculated with 5 millimetre piece of stock culture of fungus under sterile conditions and incubated over a period of 4 days at 37^0C. The inhibition of fungus growth was observed and measured in radial direction by using distance measurement scale.

Calculation:

Inhibition of radial growth was computed based on colony diameter on control plate using the following formulaas stated by Sundar *et al.* (1995).

$$\% \text{ inhibition} = \frac{X - Y}{X} \times 100$$

where,

X= Growth of control plate

Y= Growth of fungicide treated plate.

Selection of plants for the study:

Nine plants were selected for assessing of antifungal activity on *F. oxysporum* species as *Azadirachta indica* (*Neem*), *Justicia adhatoda* (Adulsa), *Carica papaya* (Papaya), *Ricinus communis* (Castor), *Ficus religiosa* (Peepale), *Ficus benghalensis* (Banyan), *Psidium guajava* (Guava), *Tamarindus indica* (Tamarind), *Annona reticulate* (Custard apple) etc.

RESULTS AND DISCUSSION

Management of Fusarium wilt is of paramount of importance in order to avoid loss associated with the infection of plants. Due to such pathogens the overall yield of the lentil decreases drastically, in order to deal this problem cost effective ecofriendly solutions need to be developed by using local resources. Present study addresses the development of solutions from ethanolic extracts of plants. As from the observations 9 plants were screened to assess their antifungal activity which are listed in Table 1.

From the observations more amount of growth was inhibited by 25 per cent extracts of almost 7 plants namely *Azadirachta indica* (*Neem*), *Justica adhatoda* (adulsa), *Carica papaya* (papaya), *Ricinus communis* (castor), *Ficus religiosa* (peepale), *Ficus benghalensis* (banyan), *Tamarindus indica* (tamarind) shown 85 per cent inhibitory effects and two plants namely *Psidium guajava* (Guava) and *Annona reticulate* (Custard apple) shown less inhibitory effects as 75 per cent and 25 per cent, respectively. 20 per cent extracts of the *Azadirachta indica* (*Neem*) and *Justica adhatoda* (Adulsa) showed 85 per cent inhibition and plant extract of peepal showed 82 per cent inhibition, while five plants namely *Carica papaya* (papaya), *Ricinus communis* (castor), *Ficus benghalensis* (banyan), *Psidium guajava* (Guava), *Tamarindus indica* (tamarind) showed 71 per cent inhibitory effects and catord apple showed 35 per cent inhibitory effects. 15 per cent plant extract of *Justica adhatoda* (adulsa) showed maximum inhibitory effect as 85 per cent, near to it, *Azadirachta indica* (*Neem*) showed about 78 per cent inhibitory effect and *Ficus religiosa* (peepale), *Ficus benghalensis* (banyan) and *Tamarindus indica* (tamarind) shows 64 per cent inhibition, other palnts such as *Carica papaya* (papaya), *Ricinus communis* (castor), *Psidium guajava* (Guava) (57%), while 28 per cent inhibitory conc was found. 10 per cent etract of *Azadirachta indica* (*Neem*) showed maximum growth inhibition of 71 per cent bellow to it *Justica adhatoda* (adulsa) showed 64 per cent inhibition and other plants were less effective as *Carica papaya* (papaya) 42 per cent, *Ricinus communis* (castor) 28 per cent, *Ficus religiosa* (peepale) 50 per cent, *Ficus benghalensis* (banyan) 57 per cent, *Psidium guajava* (Guava) 50 per cent, *Tamarindus indica* (tamarind) 50 per cent and *Annona reticulate* (Custard apple) showed very less inhibition of growth as (7%). The graphical representation of the results of inhibition is as shown in the Fig 1.

From the results of the study of ethanolic extracts of 9 plants for various concentrations with growth medium for cultures of *F. oxysporum* most of the plants showed inhibitory effect of about 85 per cent in 25 per cent concentration of plant extract with the medium as described in the results except *Psidium guajava* (Guava) and *Annona reticulate* (Custard apple) plant extracts which have less inhibitory effects of 78 per cent and 35 per cent, the results are very effective against fungal species of *F. oxysporum*. 20 per cent extracts of plants such as *Azadirachta indica* (*Neem*), *Justica adhatoda* (adulsa), *Ficus religiosa* (peepale), *Carica papaya* (papaya) *Ricinus communis* (castor), *Ficus benghalensis* (banyan) *Psidium guajava* (Guava) and *Tamarindus indica* (tamarind) showed less effective as compared to 25 per cent extracts but also inhibited about more than 70 per cent growth of the fungus except

Table 1: Inhibition of growth by various plant extracts

Sr.No.	Plant name	Percentage of extract	Control growth in cm	Growth in cm
1.	*Azadirachta indica*	10 %	7	2
	(*Neem*)	15 %	7	1.5
		20%	7	1
		25 %	7	1
2.	*Justicia adhatoda*	10 %	7	2.5
	(Adulsa)	15 %	7	1
		20%	7	1
		25 %	7	1
3.	*Carica papaya*	10 %	7	4
	(Papaya)	15 %	7	3
		20%	7	2
		25 %	7	1
4.	*Ricinus communis*	10 %	7	5
	(Castor)	15 %	7	3
		20%	7	2
		25 %	7	1
5.	*Ficus religiosa*	10 %	7	3.5
	(Peepale)	15 %	7	2.5
		20%	7	1.2
		25 %	7	1
6.	*Ficus benghalensis*	10 %	7	3
	(Banyan)	15 %	7	2.5
		20%	7	2
		25 %	7	1
7.	*Psidium guajava*	10 %	7	3.5
	(Guava)	15 %	7	3
		20%	7	2
		25 %	7	1.5
8.	*Tamarindus indica*	10 %	7	3.5
	(Tamarind)	15 %	7	2.5
		20%	7	2
		25 %	7	1
9.	*Annona reticulate*	10 %	7	6.5
	(Custard apple)	15 %	7	5
		20%	7	5
		25 %	7	4.5

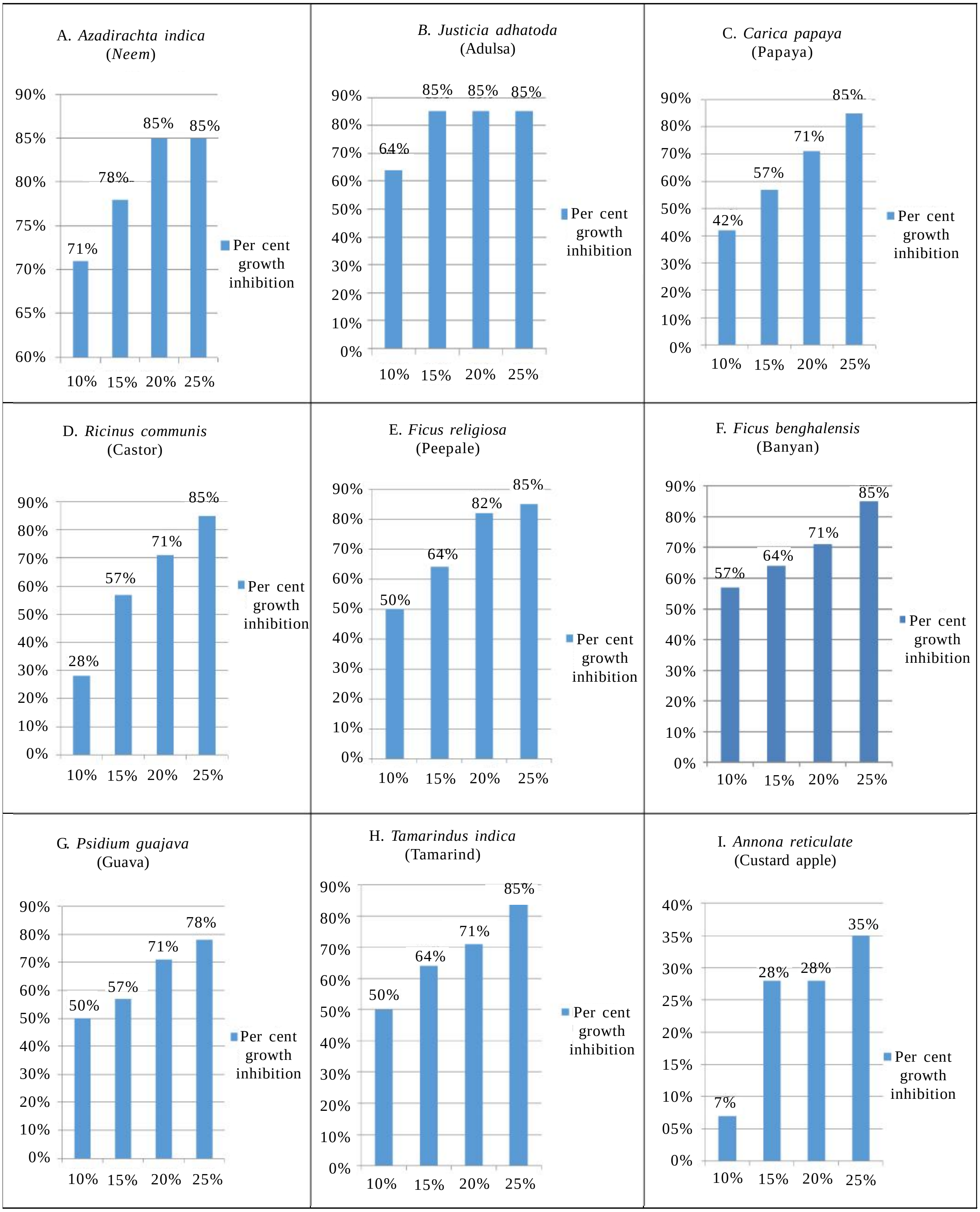

Fig. 1 : Graphical representation of percentage growth inhibition by different phytoextracts

Annona reticulate (Custard apple) which showed 28 per cent inhibitory effects. The growth of Fusarium was inhibited for about more than 55 per cent by eight plants except 28 per cent by the *Annona reticulate* (Custard apple). 10 per cent plant extracts of *Azadirachta indica* (*Neem*) and *Justica adhatoda* (adulsa) showed maximum growth inhibition of greater than 60 per cent as compared to other plants, the inhibitiory effects of more than 40 per cent was shown by *Ficus religiosa* (peepale), *Ficus benghalensis* (banyan), *Psidium guajava* (Guava), *Tamarindus indica* (tamarind) and *Carica papaya* (papaya), but very less effect of growth inhibition was reported as 28 per cent by *Ricinus communis* (castor) and 7 per cent by *Annona reticulate* (Custard apple).

Thus, from above points it can be concluded that various concentration of about eight plant extracts namely above 15 per cent showed maximum growth inhibitory effects and can be effectively utilised as an bio control agent for controlling the adversed effects of these pathogens on overall crop yields. The overall effects of these antifungal extracts can be confirmed by assessing field trials of these extracts on affected farms or by performing pot experiments.

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