The Pharma Innovation Journal 2019; 8(8): 297-302

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(8): 297-302

www.thepharmajournal.com
Received: 16-06-2019
Accepted: 18-07-2019

M Sowmya Vani
Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya-Jabalpur, Madhya Pradesh, India

Sanjeev Kumar
Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya-Jabalpur, Madhya Pradesh, India

Rahul Gulya
Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya-Jabalpur, Madhya Pradesh, India

Correspondence
Sanjeev Kumar
Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya-Jabalpur, Madhya Pradesh, India

In vitro evaluation of fungicides and plant extracts against *Fusarium oxysporum* causing wilt of mungbean

M Sowmya Vani, Sanjeev Kumar and Rahul Gulya

Abstract
Of late, the incidence of wilt of mungbean caused by *Fusarium oxysporum* has increased substantially in the mungbean growing areas of Madhya Pradesh. *In vitro* studies were conducted to evaluate the efficacy of seven different fungicides *viz.*, Azoxystrobin, Propineb, Thiophanate Methyl, Difenoconazole, Mancozeb, Mancozeb+Thiophanate methyl, Boscolid+Pyraclostrobin and locally available plants *viz.*, *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Parthenium hysterophorus*, *Polyalthia longifolia*, *Ricinus communis* and *Withania somnifera* against *Fusarium oxysporum.* Mancozeb+Thiophanate methyl (0.15%), was the best fungicide which completely inhibited the growth and sporulation of the fungus, followed by Propineb, Mancozeb, Boscolid+Pyraclostrobin, Difenaconazole. Parthenium leaf extract was found best in inhibiting the growth and sporulation of *F. oxysporum* as it produced 78.3 percent growth inhibition of *F. oxysporum* at 15 percent concentration followed by castor leaf extract (69.4 percent growth inhibition).

Keywords: Mungbean, wilt, *Fusarium oxysporum*, fungicides and plant extracts

Introduction
Mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) is one of the important pulse crops in South and Southeast Asia. India produces about 1.5–2.0 million tons of mungbean annually from about 3–4 million hectares with an average productivity of 0.5 t ha^{-1} (Jadhav *et al*., 2016) [7]. Mungbeanseeds is a good source of dietary protein for humans including marginal people, and people who live in areas with less access to meat or where people are mostly vegetarian. Mungbean sprouts and green pods contain high level of vitamins and minerals (Keatinge *et al*., 2011[9]; Nair *et al*., 2015) [14].

Abiotic and biotic stresses caused significant decline in mungbean yield India. Among biotic stresses, fungal diseases are responsible for reducing yield up to 40–60% in mungbean (Kaur *et al.*, 2011) [8]. Fungal pathogens can infect mung bean plants at different stages, such as during emergence, seedling, vegetative, reproductive stages and cause substantial damage leading to yield loss or complete failure of production.

Fusarium wilt caused by *Fusarium oxysporum* has been a minor disease of mungbean in Madhya Pradesh. However, the incidence of the disease has increased substantially in recent years in the mungbean growing areas of Madhya Pradesh. Fusarium wilts first appear as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At the seedling stage, plants infected by *F. oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and drying of young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant (Agrios, 1988) [1]. Browning of the vascular tissue is strong evidence of Fusarium wilt. Further, on older plants, symptoms generally become more apparent during the period.

Chemical fungicides are used to control Fusarium wilt in other crops. Root and stem rot can be managed by drenching soil or treating seeds with thiram or benomyl (Rose *et al.*, (2003) [19]. Thiphanate –methyl have been consistently reported to be effective against *F. oxysporum* on different host-plants (Nel *et al.*, 2007; Tarekegn *et al.*, 2007) [15, 23]. Furthermore, the efficiency of fungicide application against Fusarium wilt of mungbean has not yet been commonly established in India. Use of natural products like botanical extracts for the management of fungal disease is considered as a substitute method to synthetic fungicides, due to their less negative impacts on the human and environment health hazard or implications. In the present work, antifungal properties of seven plant extracts and fungicides against *Fusarium oxysporum.*

were evaluated to find out an effective approach for management of wilt of mungbean

Material and methods

Collection of disease sample

Mungbean plants showing characteristic symptoms of Fusarium wilt were collected from the Experimental Farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.). Infected plants showing wilt symptoms were uprooted and washed to remove the adhered soil particles. The stem and root portions were separated with sterile scalpels and kept in different envelopes. Each envelope was marked clearly mentioning location, variety, date of collection etc. and were brought to the laboratory. The samples were dried for 24 hours in shade in order to remove excess surface moisture. After drying, the samples were kept in B.O.D. incubator in paper envelop and maintained for isolation and further studies.

Isolation of pathogen

The diseased parts of mungbean samples showing distinct characteristics of wilt symptom were selected for isolation of the pathogen. The selected roots, collar region and stem were washed with fresh sterilized water in order to remove the dust particles and surface contaminants. The diseased parts were then cut into small bits with the help of sterilized scalpel. The cut pieces were surface sterilized with 0.1 per cent mercuric chloride solution under aseptic condition inside a laminar flow and washed thoroughly 3 to 4 times with sterilized water to remove the trace of mercuric chloride. Excess moisture was removed by placing these in the fold of sterilized blotting papers. The pieces were then transferred in Petri plates with the help of sterilized needles. Petri plates used in the experiment were previously sterilized and poured with Potato dextrose agar medium. Three to four dissected pieces ofdiseasedparts of mungbean were placed in Petri plates of equal distances from each other. The Petri plates were kept at 28±2ºC for 7 days in an incubator. As soon as the mycelial growth was visible, the hyphal tips from the advancing mycelium were cut and transferred into the culture slants containing Potato Dextrose Agar (PDA) medium for purification, identification and maintenance of pure culture by single spore isolation technique.

Estimation of spore formation of *Fusarium oxysporum*

For estimating the sporulation, at the end of the incubation period 5 mm disc was cut and suspended in 10 ml of distilled water and shaken well to harvest spores. Numbers of spores were counted with the help of Haemocytometer. The results have been expressed as excellent, good, fair, poor, and no sporulation on the basis of the following scale.

Table 1: Details of expression of sporulation

Sporulation	Represented as	No. of spores/ microscopic field
Excellent	++++	61 & above
Good	+++	41 – 60
Fair	++	21 – 40
Poor	+	Less than 20
No	–	–

Evaluation of fungicides

The experiment was laid out in Completely Randomized Design (CRD) with eight treatments and three replications. Seven individual and combination fungicides like Azoxystrobin (0.1%), Propineb (0.2%), Thiophanate Methyl (0.1%), Difenoconazole (0.1%), Mancozeb (0.2%), Mancozeb + Thiophanate methyl (0.15%), Boscolid+Pyraclostrobin (0.1%) along with the control were evaluated against *Fusarium oxysporum* under laboratory conditions to screen out the best fungicides upon their inhibitory effect on the growth. The efficacy of fungicides was tested against the pathogen by "Poisoned food Techniques" (Mortan and Straube, 1955) [12]. One set of control was also kept in which the medium was not mixed with fungicides. Seven days old culture of *Fusarium oxysporum* was cut with the help of 5/6 mm cork borer was inoculated in each Petri dish at the center. These inoculated Petri-dishes were incubated at 28+1^{0}C. The fungal growth was recorded in each petriplate after 168 hours of the incubation. The recorded data on radial growth was converted into percent growth inhibition by using following formula given by Vincent, 1947 [24].

Evaluation of plant extracts

Seven locally available plants *viz.*, *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Parthenium hysterophorus*, *Polyalthia longifolia*, *Ricinus communis* and *Withania somnifera* were tested for their antifungal activity against *Fusarium oxysporum.* Extracts of plant parts such as leaf, bulb and clove etc. were prepared by the standard method used by Gerard *et al*. (1994) [4]. Fresh plant parts were washed with tap water followed by sterile distilled water, processed with sterile distilled water @1mlg-1 of plant tissue (1:1v/w) with pestle and mortar and filtered through a double layered cheese cloth. The filtrate so obtained formed the standard plant extract solution. The plant extract so prepared were screened *in vitro* against *Fusarium oxysporum* using poisoned food technique. Stock solution 5, 10 and 15 ml were mixed respectively with 95, 90 and 85 ml of sterilized molten Potato Dextrose Agar (PDA) media to obtain 5, 10 and 15 percent concentration of plant extract. The mixed medium was thoroughly shaken to ensure uniform mixing of extract. 20 ml of poisoned PDA was poured into sterile petriplates. Three replications were maintained for each concentration. After solidification of poisoned media, the plates were inoculated with mycelium disc (5 mm diameter) of vigorously growing pure culture colony of *Fusarium oxysporum.* The control petriplates in three replications were maintained using PDA without any plant extract with mycelium disc (5 mm) for comparison. Plates were incubated at 28 ± 1ºC and observation on radial growth after 120 and 196 hours and sporulation after 15 days of test fungus was recorded. Recorded data on radial growth and sporulation was converted into percent inhibition by using following formula given by Vincent, 1947 [24].

$$\text{Percent growth inhibition (I)} \quad \frac{C - T}{C} \quad X100$$

Where,
C = Radial growth in check plate (mm)
T = Radial growth in the treated plate (mm)

Result and Discussions

Evaluation of fungicides

It is evident from the result of Table-2 that all the fungicide significantly inhibited the radial growth and sporulation of *Fusarium oxysporum*.

However, combination of Mancozeb +Thiophanate methyl

(0.15%) were found best fungicide which completely inhibited the radial growth of *Fusarium oxysporum* after 168 hrs of incubation. Propineb (0.2%), Mancozeb (0.2%), Boscolid+ Pyraclostrobin (0.1%), Difenaconazole (0.1%) were second next in order of toxicity percent inhibition of radial growth. Least inhibition was recorded in Azoxystrobin (55.8%). No sporulation was observed in Mancozeb + Thiophanate Methyl fungicide (Fig. 1 & 2). Poor sporulation was the observed in Propineb, Mancozeb, Boscolid + pyraclostrobin fungicides, while fair sporulation was recorded in Thiophante methyl, Difenaconazole and Azoxystrobin. Observed results are in accord with the findings reported by Rajput *et al*., (2006) [18], Mukhtar (2007) [13], Khan *et al*., (2012) [10], Sultana &Ghaffar (2010) [22] and Sangeetha and Jahagirdar (2013) [21] Maitlo *et al.* (2014) [11] and Poussio *et al* (2018) [17].

Evaluation of plant extracts

Data presented in Table-3 clearly reveals that none of the plant extracts could completely inhibited the growth of *F. oxysporum* even at 15 percent concentration. Parthenium leaf extract was found very promising in inhibiting the growth of *F. oxysporum* as it produced 78.3 percent growth inhibition of *F. oxysporum* at 15 percent concentration. Castor leaf extract was found effective to some extent as they produced 69.4 percent growth inhibition at 15% concentration. Other plant extracts produced 54.5 to 66.2 percent growth inhibition at 15 per cent concentration. At lower concentrations i.e.5 and 10 per cent growth inhibition due to plant extracts were from 29.8 to 65.8 percent (Fig. 3 & 4). Botanicals used in this experiment are extensively used due to their important usage in traditional medicine, and high content of polyphenols, flavonoids, phenolic acids, tannins, quinines, coumarins, terpenoids and alkaloids present in them (Hadi*et al.* (2013) [5]; Hatamleh *et al.* (2014) [6]; Awad (2016) [3]. The mechanism expected to be responsible for toxicity against pathogens may involves various targets *viz.*, interference with the synthesis of cellular walls, alteration of cell permeability, interference with the transport of electron, the nutrient absorption, the adenosine triphosphatase and other metabolic processes of the cell, deactivation of various cellular enzymes and denaturation of cellular proteins (Al-Amiery (2012) [2]. *Allium sativum* contains flavonoids, phytic acid, tannins and phenols. Its aqueous extract promoted almost total inhibition of the mycelium.

Table 2: Effect of fungicides on radial growth and sporulation of *Fusarium oxysporum*

Treatment No.	Fungicides	Dosage (%)	Radial growth (mm) after 168 hrs*	Percent growth inhibition over check	Sporulation
T1	Azoxystrobin	0.1	25.3	55.8	+++
T2	Propineb	0.2	7.8	86.6	+
T3	Thiophanate Methyl	0.1	15.5	73.5	++
T4	Difenaconazole	0.1	11.8	79.7	+
T5	Mancozeb	0.2	9.1	84.3	+
T6	Mancozeb+Thiophanate methyl	1.5	0.0	100.0	-
T7	Boscolid+Pyraclostrobin	0.1	10.6	81.7	+
T8	Control	-	58.5	--	++++
SE(m)±			1.019		
CD (0.05)			3.081		

*Mean of three replications

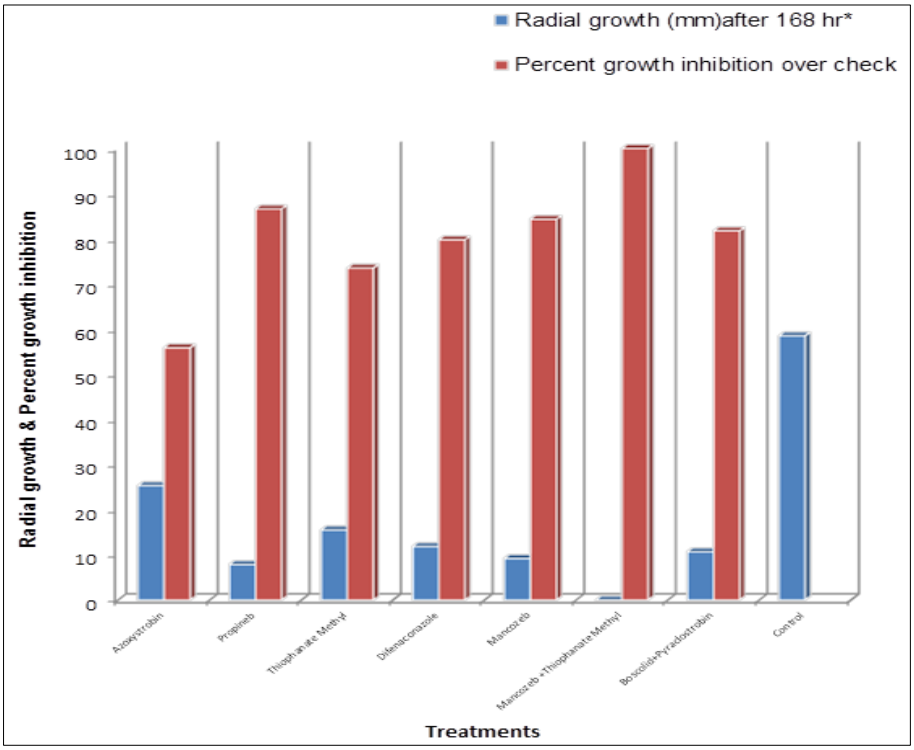

Fig 1: Effect of fungicides on radial growth and sporulation of *Fusarium oxysporum*

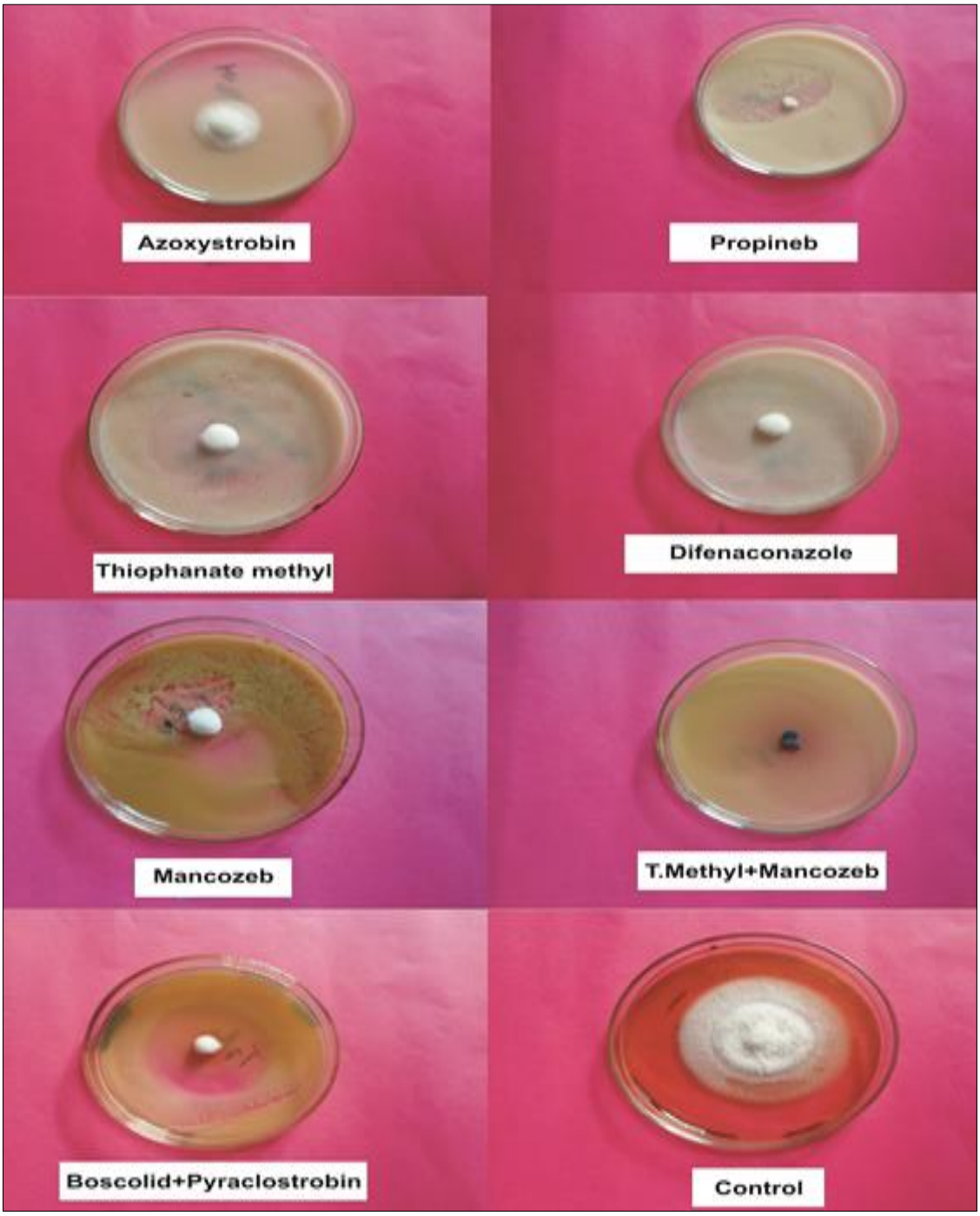

Fig 2: Inhibitory effect of different fungicides on growth and sporulation of *Fusarium oxysporum*

Table 3: Effect of plant extracts on radial growth and sporulation of *Fusarium oxysporum* after seven days of incubation

T. no.	Name of plant extracts	Local name	Parts used	Radial growth of target pathogen (mm) *			Mean	Percent growth inhibition			Sporulation		
				5%	10%	15%		5%	10%	15%	5%	10%	15%
T1	*Allium cepa*	Onion	Bulb	49.6	41.6	26.3	49.3	33.51	43.1	64.4	++++	++++	++
T2	*Allium sativum*	Garlic	Clove	32.3	37.3	27.3	32.3	56.7	49.0	63.0	++++	+++	++
T3	*Azadirachta indica*	Neem	Leaf	52.3	49.6	31.6	52.1	29.8	32.2	57.2	++++	++++	++
T4	*Parthenium hysterophorus*	Parthenium	Leaf	39.8	25.0	16.0	37.3	46.6	65.8	78.3	++++	++++	+
T5	*Polyalthia longifolia*	Ashoka	Leaf	49.0	37.6	25.0	33.7	34.3	48.5	66.2	++++	++++	++++
T6	*Ricinus communis*	Castor	Leaf	46.0	25.3	22.6	31.3	38.3	64.0	69.4	++++	+++	++
T7	*Withania somnifera*	Ashwagandha	Leaf	40.0	38.3	33.6	24.2	46.3	47.6	54.5	++++	++++	++++
T8	Control	-	-	74.6	73.0	74	43.4				++++	++++	++++
		SE(m)±		0.008	0.006	0.002							
		CD (0.05)		0.023	0.017	0.006							

*Mean of three replications

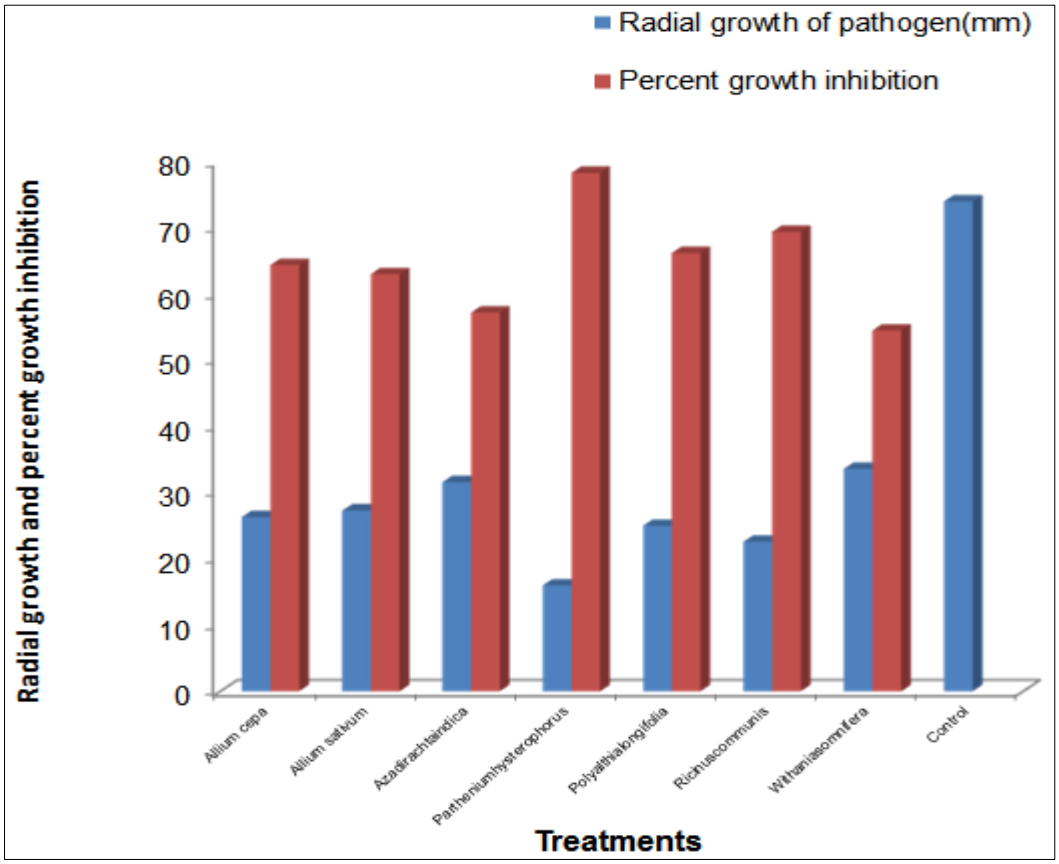

Fig 3: Effect of plant extracts on radial growth and sporulation of *Fusarium oxysporum* after seven days of incubation

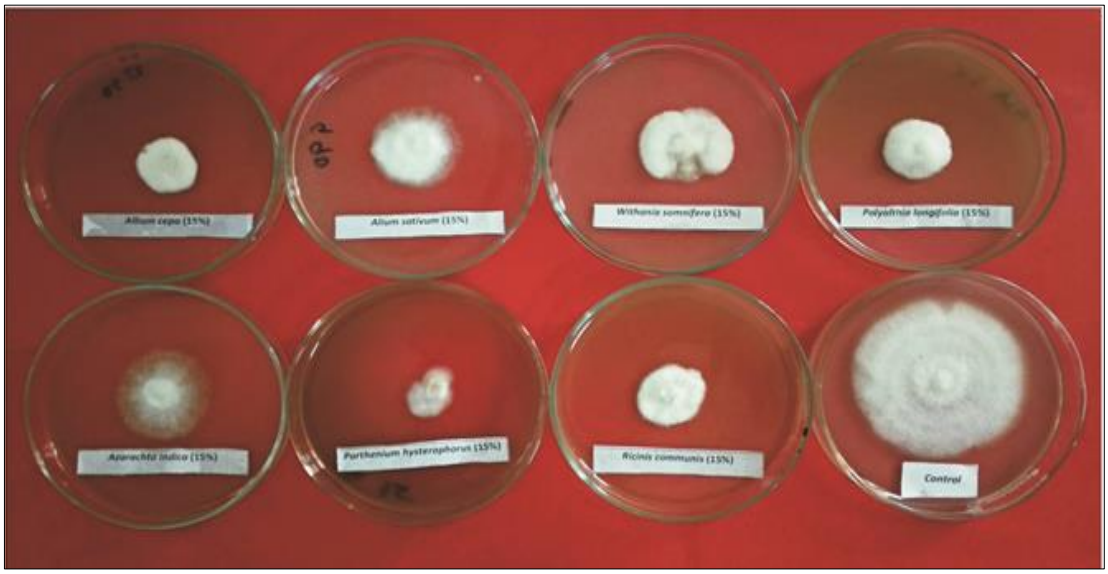

Fig 4: Inhibitory effect of plant extracts at 15% concentration on growth and sporulation of *Fusarium oxysporum*

Conclusion

It is concluded that the fungicides were found more effective as compared to plant extracts for management of Fusarium wilt disease of mungbean. The Mancozeb+Thiophanate methyl (0.15%) was found most effective fungicide and *Parthenium hysterophorus* plant leaf extract inhibited 78.3 percent growth of *F. oxysporum* at their higher concentration (15%).

References

1. Agrios GN. Plant Pathology, 3rd. ed. Academic press, Inc. New York, 1988, 803p.
2. Al-Amiery AA, Kadhum AAH, Mohamad AB. Antifungal and Antioxidant Activities of Pyrrolidone Thiosemicarbazone Complexes, Bioinorganic Chemistry and Applications, 2012. https://doi.org/10.1155/2012/795812.
3. Awad HM. Evaluation of plant extracts and essential oils for the control of sudden wilt disease of watermelon plants. International Journal of Current Microbiology and Applied Science. 2016; 5:949-962.
4. Gerard E, Chandrasekar, Kurucheve V. Effect of six selected plant products and oil cakes on the Sclerotial production and germination of *Rhizoctonia solani*. Indian Phytopathology. 1994; 47(2):183-185.
5. Hadi M, Kashefi B, Sobhanipur A, Rezaarabsorkhi M.

Study on effect of some medicinal plant extracts on growth and spore germination of *Fusarium oxysporum* schlecht- *In vitro*. American-Eurasian Journal of Agriculture & Environment Science. 2013; 13:581-588.
6. Hatamleh AA, Bahkali AH, El-SHeshtawi M, Elgorban AM, El-Metwally. 2014. Inhibitory influence of plant extracts on soil borne fungi infecting muskmelon (*Cucumis melo* L.). International Journal of Pharmacology. 2014; 10:322-327.
7. Jadhav ML, Taur N, Sapkal S, Tathe S, Quadri F. Study on effect of caffeine on growth of *Vigna radiata* L. International Journal for Advanced Research. 2016; 4:596-602.
8. Kaur L, Singh P, Sirari A. Biplot analysis for locating multiple disease resistant diversity in mungbean germplasm. Disease Research. 2011; 26:55-60.
9. Keatinge JDH, Easdown WJ, Yang RY, Chadha ML, Shanmuga Sundaram S. Overcoming chronic malnutrition in a future warming world: the key importance of mungbean and vegetable soybean. Euphytica. 2011; 180:129-141. doi: 10.1007/s10681-011-0401-6
10. Khan I, Saifulla M, Mahesh SB, Pallavi MS. Effect of different media and enviromental conditions on the growth of *Fusarium* oxysporum f.sp. *ciceri* causing *Fusarium* wilt of chickpea. International Journal of Science and Nature. 2011; 2(2):402-404
11. Maitlo SA, Syed RN, Rustamani MA, Khuhro RD, Lodhi AM. Comparative efficacy of different fungicides against *Fusarium* wilt of chickpea (*Cicer arietinum* L.) Pakistan Journal of Botany. 2014; 46(6):2305-2312.
12. Morton DT, Straube NH. Antagonistic and stimulatory effects of microorganisms *Sclerotium rolfsii* Phytopathology. 1955; 45:419-420.
13. Mukhtar I. Comparison of phytochemical and chemical control of *Fusarium oxysporum* f. sp. *ciceris*. Mycopathology. 2007; 5(2):107-110.
14. Nair RM, Thavarajah D, Thavarajah P, Giri RR, Ledesma D, Yang RY. Mineral and phenolic concentrations of mung bean [*Vigna radiata* (L.) R. Wilczek var. *radiata*] grown in semi-arid tropical India. Journal of Food Compost Annalysis. 2015; 39:23-32. doi: 10.1016/j.jfca.2014.10.009
15. Nel B, Steinberg C, Labuschagne N, Viljoen A. Evaluation of fungicides and sterilants for potential application in the management of Fusarium wilt of banana. Crop Protection. 2007; 26:697-705.
16. Poddar RK, Singh DV, Dubey SC. Management of chickpea wilts through combination of fungicides and bioagents. Indian Phytopathology. 2004; 57:39-43.
17. Poussio GB, Abro MA, Hajano JA, Khaskheli MI, Rajput KI, Memon SA. Potential of plant extracts and fungicides for managing *Fusarium oxysporum f. splycopersici* Pakistan. Journal of Phytopathology. 2018; 30(01):75-81.
18. Rajput AQ, Arain MH, Pathan MA, Jiskani MM, Lodhi AM. Efficacy of different fungicides against Fusariumwilt of cotton caused by *Fusarium oxysporum* f. sp. *vasinfectum*. Pakistan Journal of Botany. 2006; 38(3):875-880.
19. Rose S, Parker M, Punja ZK. Efficacy of biological and chemical treatment for control of Fusarium root rot and stem rot of greenhouse cucumber. Plant Disester. 2003; 87:1460e1462.
20. Ryley M, Toowoomba Tatnell J. Management of the major foliar diseases of mung bean and peanuts in Australia. Kingaroy: Agri-Science Qld © The State of Queensland, Department of Employment, Economic Development and Innovation, 2010.
21. Sangeetha TV, Jahagirdar S. Screening of new molecules of fungicides against *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium* spp. causing root rot/wilt complex of soybean. International Journal of Plant Protection. 2013; 6:90-94.
22. Sultana N, Ghaffar A. Effect of fungicides, microbial antagonists and oilcakes in the control of *Fusarium solani*, the cause of seed rot, seedling and root infection of bottle gourd, bitter gourd and cucumber. Pakistan Journal of Botany. 2010; 42(4):2921-2934.
23. Tarekegn G, Sakhuja PK, Swart WJ, Tamado T. Integrated managementof groundnut root rot using seed quality and fungicide seed treatment. International Journal of Pest Management. 2007; 53:53-57.
24. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature. 1947; 154:850.