Print ISSN: 0976-2876 Online ISSN: 2250-0138

Available online at: http://www.ijsr.in

INDIAN JOURNAL OF SCIENTIFIC RESEARCH

DOI:10.32606/IJSR.V15.I2.00001

Received: 11-10-2024 Accepted: 25-12-2024 Publication: 31-01-2025

Indian J.Sci.Res. 15 (2): 01-05, 2025 Original Research Article

IN -VITRO SCREENING OF DIFFERENT BOTANICAL EXTRACTS AGAINST *Fusarium oxysporum f. sp. ciceris* CAUSING WILT DISEASE OF CHICKPEA (*Cicer arietinum* L.)

SHILA MAURYA[a] AND SUBHASH CHANDRA[b1]

[ab]Department of Botany, Sri Murli Manohar Town P.G. College, Ballia (U.P.), India

ABSTRACT

***In- vitro* study was carried out to evaluate the effect of twenty botanical extracts against isolated endophytic pathogens *Fusarium oxysporum f. sp. ciceris* which causes serious wilt disease in Chickpea. Twenty botanical extracts have been accounted in studies and depicited as treatment(T) as T1- Neem leaf extract, T2- Lemon leaf extract, T3- Dhatura leaf extract, T4- Garlic bulb extract, T5-Ginger rhizome extract, T6-Laung leaf extract, T7-Tulsi leaf extract, T8-Peepal leaf extract, T9-Karripatta leaf extract, T10-Bel leaf extract, T11-Castor leaf extract, T12-Jamun leaf extract, T13-Sadabahar leaf extract, T14- Arjun leaf extract, T15-Sahajan leaf extract, T16-Mahogany leaf extract, T17-Neem fruit extract, T18-Kadam leaf extract, T19- Chhitwan leaf extract, T20-Anar leaf extract, in different concentrations 5%, 10%, 15% respectively. The result of the study was concluded in terms of % inhibition against control by examine radial growth of test fungus on treatments. Maximum % inhibition was exhibited in T1- Neem leaf and T17-Neem fruit whereas minimum % inhibition were exhibited in T18-Mahogany leaf extracts.**

KEYWORDS: *Fusarium oxysporum f.sp.ciceris*, Chickpea, Fusarium wilt, Botanical extracts

Among all the Pulses, Chickpea (*Cicer arietinum* L.) commonly knows as Black Gram is the world's fourth most important *Rabi* season legume crop rich in highly proteinous nutrients and a good source of vitamins, minerals and fibre. Chickpea also has advantages in the management of soil fertility, Particularly in dry lands and semiarid tropics (Singh and Saxena, 1996). But the average global productivity of Chickpea is constrained due to several biotic (viruses, bacterial and fungal pathogens) and abiotic (pH level, salinity, water holding capacity and electric conductivity) factors. (Reddy *et al*., 1990; Tarafdar *et al*., 2017, 2018).Due to susceptibility to biotic factors viz several fungal, bacterial and viral diseases low yields of Chickpea are attributed in many cropping regions. Among the disease affecting Chickpea, vascular wilt is commonly found and it causes severe downfall in the production of legumes plant. The disease occured due to an important endophytic fungus *Fusarium oxysporum f. sp. ciceris.* The Disease was first appeared and reported in Panama canal, Australia as stated by Ploetz and Pegg (1997). In Indian subcontinent, Fusarium wilt was first reported in West Bengal (Niwas *et al*., 2021). The disease is more prominent in the Indian subcontinent, Spain, Ethiopia, Mexico, Tunisia, Turkey and United state (Westerlund *et al.,* 1974, Halila and Strange 1996; Ghosh *et al*., 2013).

Fusarium wilt epidemics causes significant annual losses of Chickpea yield which account for 10 to 15 % of total yields and sometimes escalate to 100 % under conditions favourable for disease (Navas- Cortes *et al.,* 2000). The disease can affect the crop at any stage of growth. Characteristic symptoms are sudden drooping of leaves and petiole and black discolouration involving xylem and pith (Dubey and Singh 2004).

Singh *et al*., (2007) reported that the fungus *Fusarium oxysporum f. sp. ciceris* can survive as mycelium and chlamydospores in seed and soil and also an infected crop residues, root and stem tissue buried in soil for upto 6 years.

Management of Fusarium wilt is a difficult task for farmer's in field. Although some strategies are use to overcome the disease like crop rotation, use of resistant varieties of Chickpea, use of different fungicides at different concentration. These strategies are not sufficient to overcome the Fusarium wilt because its ability to survive many years in soil in forms of chlamydospore and application of excess fungicide also degrade the soil texture and soil fertility and moreover resistant varieties are also not effective against different races of the pathogen.

Therefore disease management through botanical extracts be a solution to control the growth of pathogen to maintain plant health. Various researcher also interpreted that botanical extracts against Fusarium wilt are very effective and also perform significant role to increase soil fertility. Botanical extracts are ecofriendly

[1]**Corresponding author**

causes no harm to environment and easily available to farmer's. The awareness of botanical extract in form of fungicides is increasing in demand and some plant products are being used globally as green fungicides (Haware *et al* 1982; Gurjar *et al.,* 2013). The present research is done to screen of various botanical extracts against management of Fusarium wilt to find out most effective botanicals in *in-vitro* conditions.

MATERIALS AND METHODS

Isolation and Purification of Pathogen

Infected Chickpea Plant that showing disease symptoms was collected from different field of Ballia District. Infected Chickpea root and shoot was rinse thoroughly with tap water and then cut into small pieces 1-2 cm. Pieces of root and shoots was surface sterilized with 0.1% mercuric chloride for 30 seconds and gently rinse three times with distilled water and dried on sterilized filter paper. After this Chickpea pieces was plated on PDA Petri plates and kept in BOD incubator at 25±2°C upto 4-5 days for incubation. After 4-5 days fungal colony was appear. Fungal colony was observed under microscope and identified on the basis of their micro and macro conidia. Pure culture was maintained by subculture the fungal colony on PDA slants and preserve at 4 °C in refrigerator.

Preparation of Botanical Extracts To Check The Efficacy Against *Fusarium oxysporum f.sp. ciceris:* Selected botanicals for screening are-

Table 1: List of selected Botanicals

S.No.	Plant Name	Botanical Name	Family Name
1.	Neem	*Azadirachta indica*	Meliaceae
2.	Lemon	*Citrus lemon*	Rutaceae
3.	Dhatura	*Datura stramonium*	Solanaceae
4.	Garlic	*Alium sativum*	Liliaceae
5.	Ginger	*Zingiber officinalis*	Zingiberaceae
6.	Laung	*Syzygium aromaticum*	Myrtaceae
7.	Tulsi	*Ocimum sanctum*	Lamiaceae
8.	Peepal	*Ficus religiosa*	Moraceae
9.	Karripatta	*Murraya koenigii*	Rutaceae
10.	Bel	*Aegle marmelos*	Rutaceae
11.	Castor	*Riccinus communis*	Euphorbiaceae
12.	Jamun	*Syzygium aromaticum*	Myrtaceae
13.	Sadabahar	*Catharanthus roseus*	Apocynaceae
14.	Arjun	*Terminalia arjuna*	Combretaceae
15.	Sahajan	*Moringa oleifera*	Moringaceae
16.	Mahogany	*Swietenia macrophylla*	Meliaceae
17.	Kadam	*Neolamarckia cadamba*	Rubiaceae
18.	Chhitwan	*Alostonia scholaris*	Apocynaceae
19.	Anar	*Punica granatum*	Punicaceae

Collected fresh leaves and bulbs of selected botanicals, washed with tap water and crushed into pre-sterile mortor and pestle with added equal amount of distilled water to fresh leaves for homogenized solution. The homogenized botanicals extract was filtered through muslin cloth and obtained filtrate considered as 100% standard extracts solution. Collected botanical extracts were added into PDA media to prepare 5%, 10%, 15% concentration respectively in 250 ml conical flask and extract were sterilized through autoclaving at 15 psi for 15 minutes and sterilized media having extracts in different concentration as given above were poured into pre-sterilized petri plates under aseptic condition. PDA containing petri plates without botanical extracts is considered as control. These petri plates (with and without botanical extracts) were inoculated with freshly cultures of *Fusarium oxysporum f. sp. ciceris* and kept in BOD incubator at 25±2℃ for 5-7 days. Three replicates were maintained of each treatment. Observation in form of radial colony growth were examined against control

after 5 days till full growth occurred in control. Result was depicted in form of % inhibition calculated by using standard formula (Vincent, 1947.)

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

Where,
I= % inhibition

C= Radial growth of Pathogen in control plates

T= Radial growth of pathogen in treated plates

Stastical Analysis

Data of fungal colony growth was recorded on 5, 7 days interval and compared with control fungal growth, Data was analysed using factorial analysis of variance(Anova) stastics software. On the basis of difference in colony growth of control and treated petri plates the percentage inhibition of pathogen was calculated using above formula.

RESULTS AND DISCUSSION

Fusarium oxysporum f. sp. *ciceris* radial colony growth is inhibited by different treatment of botanical extracts at 5%, 10%, 15% concentration on PDA media. At 5% and 10% concentration maximum percent inhibition was exhibited in T1- Neem leaf extract (*Azadirachta indica*) (85.60%), and T17-Neem fruit (82.22%), followed by T9- Karripatta leaf (*Murraya koenigii*) (75.39%), T4–Garlic bulb*(Alium sativum*) (73.72%), T5-Ginger (*Zingiber officinalis*) (71.67%) least inhibition of test fungus was exhibited in T15- Sahajan leaf (*Moringa oleifera*) (45.39%), and T16-Mahogany leaf (*Swietenia macrophylla*) (41.11%). Similarly in 15% concentration maximum percent inhibition was exhibited by T1- Neem leaf (87.94%), and T17-Neem fruit (85.17%), followed by T9 – Karripatta leaf (77.39%), T4- Garlic bulb (77.06%), T5- Ginger rhizome (71.83%), and least inhibition were exhibited by T14-Peepal leaf (49.28%), T11- Castor leaf (47.78%), T15- Sahajan leaf (47.22%), T16-Mahogany leaf (43.50%). From the result observed it is cleared that maximum inhibition of test fungus is noted in Neem and Karripatta extracts followed by Garlic bulb and Ginger rhizome extracts. Although there is marginal difference in percent inhibition of Garlic bulb and Ginger rhizome extracts at three concentration in studies and least inhibition at three concentration is observed in Sahajan and Mahogany extracts. From the study it is concluded that maximum inhibition of test fungus in form of mycelia growth is obtained by using Neem extracts. So, if we implement the extracts in soil alongwith the Chickpea seeds. The seed will remain free from pathogenic test fungus. Various researchers also obtained similar results by using Neem extracts to check the mycelia growth of test fungus at different concentration (Benkeblia, 2004; Hassanein *et al*., 2008; Banso *et al*., 2009). Singh *et al.* (1980) and Mukhtar (2007) also reported that Aqueous leaf extracts of Neem is highly effective is reduced the mycelia growth of test fungus. Neem extracts causes deformation of the mycelium, vacoulation of the mycelia cytoplasm and herniation of the cytoplasmic contents. (Abyaneh *et al*., 2005). The deterioration of *Fusarium oxysporum f. sp.ciceris* by Neem extracts is due to Azadirachtin antifungal compound and nimbin, nimbdin and salannin (Lale and Abdulrahman, 1999). Besides Neem extracts the effectiveness of Karripatta and Garlic bulb extract also exhibit significant retardation in radial growth of test fungus. Although best retardation was exhibited by Karripatta followed by Garlic bulb extracts. Several researchers also reported that Garlic bulb extracts is also a potant inhibitor at high concentration to test fungus, but none of the report on Karripatta is recorded.

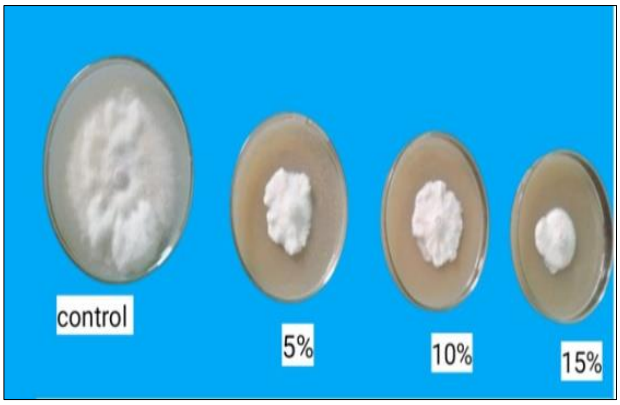


Figure 1: Neem fruit against *Fusarium oxysporum f.sp. ciceris*

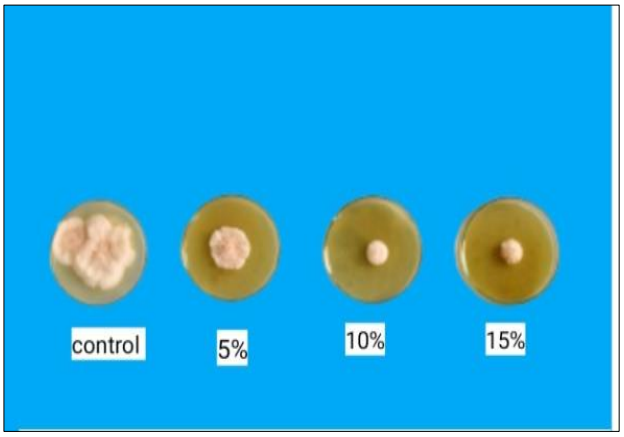
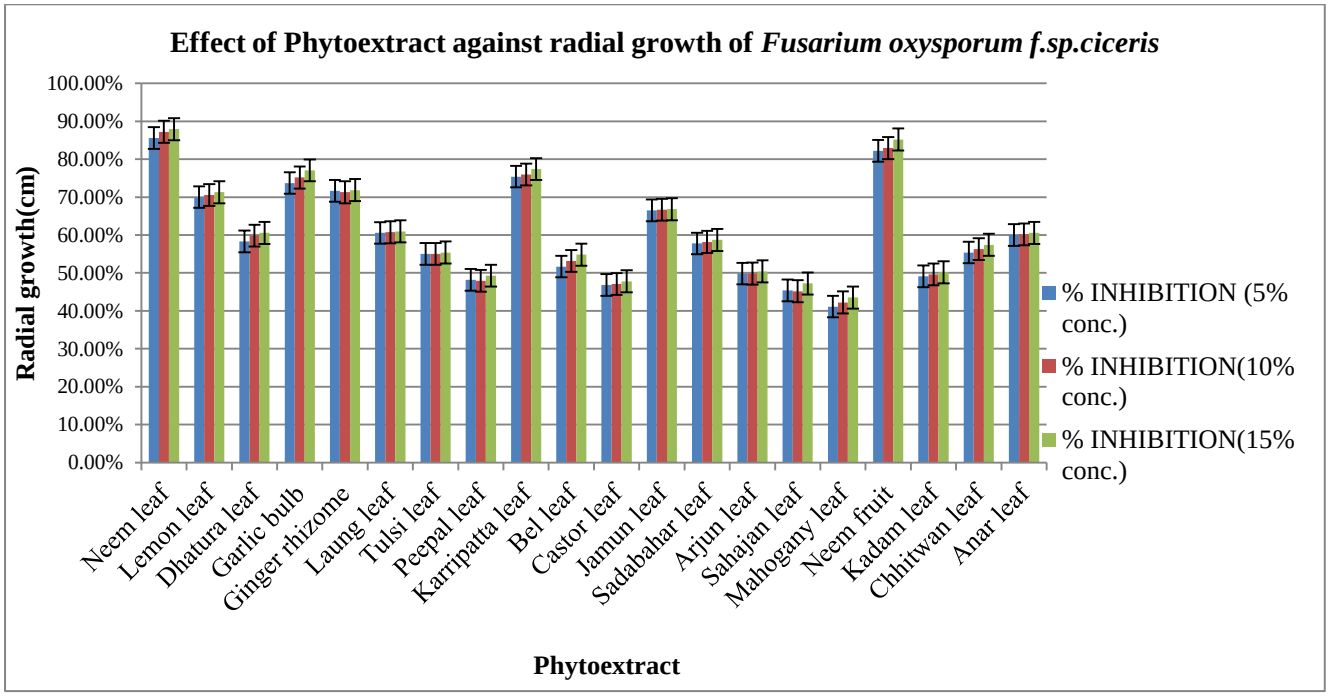


Figure 2: Neem leaf extract against *Fusarium oxysporum f.sp. ciceris*

Table 2: Effect of selected botanicals on radial growth of *Fusarium oxysporum f.sp. ciceris*

S.N.	Treatement	Mean (5%)	Mean (10%)	Mean (15%)	SD1 (5%)	SD2 (10%)	SD3 (15%)	Inhibition (5%)	Inhibition (10%)	Inhibition (15%)
1	Neem leaf	2.60	2.30	2.17	0.36	0.30	0.29	85.60%	87.22%	87.94%
2	Lemon leaf	5.40	5.30	5.17	0.53	0.52	0.29	70.00%	70.56%	71.28%
3	Dhatura leaf	7.50	7.23	7.10	0.46	0.12	0.10	58.33%	59.83%	60.56%
4	Garlic bulb	4.73	4.47	4.13	0.38	0.15	0.06	73.72%	75.17%	77.06%
5	Ginger rhizome	5.10	5.17	5.07	0.17	0.06	0.06	71.67%	71.28%	71.83%
6	Laung leaf	7.10	7.07	7.03	0.10	0.12	0.06	60.56%	60.72%	60.94%
7	Tulsi leaf	8.10	8.10	8.03	0.17	0.10	0.06	55.00%	55.00%	55.39%
8	Peepal leaf	9.33	9.37	9.13	0.31	0.12	0.06	48.17%	47.94%	49.28%
9	Karripatta leaf	4.43	4.33	4.07	0.15	0.15	0.06	75.39%	75.94%	77.39%
10	Bel leaf	8.70	8.43	8.13	0.10	0.21	0.06	51.67%	53.17%	54.83%
11	Castor leaf	9.57	9.53	9.40	0.12	0.06	0.10	46.83%	47.06%	47.78%
12	Jamun leaf	6.03	6.00	5.97	0.15	0.10	0.15	66.50%	66.67%	66.83%
13	Sadabahar leaf	7.60	7.53	7.43	0.52	0.06	0.06	57.78%	58.17%	58.72%
14	Arjun leaf	9.03	9.03	8.93	0.06	0.12	0.06	49.83%	49.83%	50.39%
15	Sahajan leaf	9.83	9.87	9.50	0.21	0.06	0.10	45.39%	45.17%	47.22%
16	Mahogany leaf	10.60	10.40	10.17	0.26	0.10	0.06	41.11%	42.22%	43.50%
17	Neem fruit	3.20	3.07	2.67	0.26	0.12	0.29	82.22%	82.94%	85.17%
18	Kadam leaf	9.16	9.07	8.93	0.29	0.12	0.06	49.11%	49.61%	50.17%
19	Chhitwan leaf	8.03	7.87	7.67	0.06	0.15	0.06	55.39%	56.28%	57.39%
20	Anar leaf	7.20	7.17	7.10	0.06	0.12	0.17	60.00%	60.17%	60.56%



Graph 1: Phytoextract against radial growth of *fusarium oxysporum f. sp. ciceris*

ACKNOWLEDGEMENT

Author is thankful to Principal, Sri Murli Manohar Town P.G. College, Ballia for providing necessary facilities and cooperation.

REFERENCES

Abyaneh M., Allameh A., Al- Tiraihi T. and Shams M., 2005. Studies on the Mode of Action of Neem (*Azadirachta indica)* Leaf and seed extracts on Morphology and Aflatoxin Production Ability of *Aspergillus parasiticus*. Acta Hortic., **1**:123-127.

Banso A., 2009. Effect of extract of *Monodora myristica* and *Zingiber officinale* on the growth of fungi in sweet potato juice. Afr. J. Bot. (in press.)

Benkeblia N., 2004. Antimicrobial activity of essential oil extracts of various onions (*Alium cepa)* and garlic (*Alium sativum*). Lebensm.- Wiss.u-Technol., **37**:263-268.

Dubey S.C. and Singh B., 2004. Reaction of chickpea genotype against *Fusarium oxysporum f. sp.ciceris* causing vascular wilt. Indian Phytopatho., **57**: 233-237.

Ghosh R., Sharma M., Telangre R. and Pande S., 2013. Occurrence and Distribution of chickpea disease in central and southern parts of india. AM. J. Plant. Sci., **4**: 940-944.

Gurjar M.S. Ali S., Akhtar M. and Singh K.M., 2012. Efficacy of Plant extract in plant disease management. Agricultural Sciences, **3**(3): 425-433.

Halila M.H. and Strange R.N., 1996. Identification of causal agent of wilt of chickpea in Tunisia *as fusarium oxysporum f. sp. ciceris* race 0. Phytopath. Mediterr., **35**: 67-74.

Hassanien N.M., Abou Zeid M.A., Youssel K.A. and Mahmood D.A., 2008. Efficacy of leaf extracts of Neem *(Azadirachta indica*) and chinaberry (*Melia azedarach*) against early blight and wilt disease of tomato. Aust.J. Basic Appl. Sci., **2**: 763-77.

Haware M.P. and Nene Y.L., 1982. Races of *Fusarium oxysporum f. sp. ciceri*. Plant Diseases, **66**: 809-810.

Lale N.E.S. and Abdulrahman H.T., 1999. Evaluation of neem (*Azadirachta indica* A. Juss) seed oil obtained by different methods and neem powder for the management of *Callosobbruchus maculates* (F.) (coeloptera: Burchidae) in stored cowpea. J. stored Peod. Res., **35**:135-143.

Mukhtar, 2007. Comparison of phytochemical and chemical control of *Fusarium oxysporum f.sp. ciceri*. Mycopath, **5**:107-110.

Navas-cortes J.A., Hau B., Jimenez-Diaz R.M., 2000. Yield loss in chickpea in relation to development of Fusarium wilt epidemics. Phytopathology. **90**: 1269-1278.

Niwas R., Chand G. and Gupta R.N., 2021. Fusarium Wilt: A Destructive Disease of Banana and Their sustainable Management. In (Ed.), Fusarium- An overview of the genus. Intech Open.

Ploetz R. and Pegg K., 1997. Fusarium wilt of banana and Wallace's line: Was the disease originally restricted to his Indo-malayan region Australian plant pathology, **26**(4): 239-249.

Reddy M.V., Nene Y.L., Singh G. and Bashir M., 1990. "Strategies for management of foliar disease of chickpea," in Proceeding of the second International Workshop on "Chickpea Improvement" (Patancheru: International cops Research Institute for the semi Arid Tropics), pp.117-127.

Singh K.B. and Saxena M.C., 1996. Winter Chickpea in Mediterranean type environments. International centre for Agricultural Research in Dry Areas. Aleppo, Syria.

Singh G., Chen W., Rubiales D., Moore K., Sharma Y.R. and Gan Y., 2007. Disease and their management. In Chickpea Breeding and management (Eds Yadav, Redden, Chen and Sharma). CAB International, pp. 497-519.

Singh U.P., Singh H.B. and Singh R.B., 1980. The fungicidal effect of neem (*Azadirachta indica)* extracts on some soil-borne pathogens og gram (*Cicer arietinum* L.). Mycologia, **72**(6): 1077-1093.

Tarafdar A., Rani T.S., Chandran U.S.S., Ghosh R. and Sharma M., 2017. "Impact of moisture stress on collar rot *(Sclerotium rolfsii* sacc.) development in Chickpea (*Cicer arietinum* L.),"in Proceedings of ISMPP International Conference on "Plant Health for Human Welfare" (Jaipur: Indian society of Mycology and Plant Pathology), 119

Vincent J.M., 1947. Distortion of fungal hyphae in presence of certain inhibitors. Nature, pp.150:850.

Westerlund F.V., Campbell R.N. and Kimble K.A., 1974. Fungal root rot and wilt of Chickpea in California. Phytopathology, **64**: 632-635.