Tagetes spp.-A Probable Potent Bio-agent to Control *Fusarium* Diseases.

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Abstract **:** Present study was done to control pathogenic *Fusarium spp.*, the most frequently occurring soil borne fungal- pathogens on vegetable crops. Every year, due to this pathogen only, there is a significant yield losses and so causing economical problems for growers and therefore the problem deserves effective measures of control. *Fusarium* control through chemical fungicides causes serious environmental problems and are toxic to non-target organisms as well. Plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumer. In our study, in-vitro antifungal efficacy of leaf extract (aqueous and ethanolic) of *Tagetes erecta* was tested against *Fusarium solani* and *Fusarium moniliforme* (isolated from soil through serial dilution culture technique) through poisoned food technique and was compared with leaf extracts of *Azadirachta indica* for five different concentrations (10%, 20%, 30%, 40% and 50%).The assessment of fungi-toxicity was carried out in terms of percentage mycelial growth inhibition against the test fungi. For both the plants, Ethanolic extracts were found to be more effective than the aqueous one. Ethanolic extract of *Tagetes erecta* was found to be the most efficient showing excellent inhibitory effect, even 100% inhibition of mycelial growth at 50% concentration for both the studied *Fusarium* spp in comparison to that of *Azadirachta indica*, 70.83% for *Fusarium solani* and 72.77% for *Fusarium moniliforme*. The results indicate that some common plants like marigold (*Tagetes* spp.), which is one of the most leading commercial floriculture crop, cultivated extensively throughout the country, could be exploited in developing a potent plant based fungicides which can be used in organic farming for the eco-friendly management of *Fusarium* species and there is a possibility of 100% replacement of the use of chemical fungicide.

Index Terms **- Leaf extracts, *Fusarium solani, Fusarium moniliforme, Tagetes, Azadirachta indica,* Poisoned food technique.**

1. Introduction

India is one of the largest vegetable producer in the world. During the last two decades considerable emphasis has been laid on increasing production of vegetable crops in India [1]. The major factors responsible for low production of vegetables are the diseases caused by soil borne pathogens, as these disease are difficult to predict, detect and diagnose. *Fusarium* species are the best known soil borne plant pathogens in terms of economical damage in agricultural productions all over the world [2] – [4]. Dry rot on potato, wilting and decline on bean or pea, crown rot and head blight on wheat, bakanae disease on rice, caused by *Fusarium* species, result to yield losses in most crop fields [5]. *Fusarium solani* (Mart.) Sacc., a soil inhabiting pathogen, attacks a

large number of host plants, including oilseeds, pulses, vegetables and ornamentals [6] – [9]. *Fusarium* dry rot of seed tuber can reduce crop establishment by killing developing potato, where crop losses can be up to 25%, while more than 60% of tubers can be infected in storage [10]. Many methods such as chemical, cultural and biological techniques have been developed for the control of plant disease by soil borne pathogens [11]. The efforts were made in other regions to manage the *Fusarium* species through phytoextracts [12] – [16] in various crops.

Tagetes erecta, commonly known as marigold, is a herbaceous, beautiful plant and is also a multipurpose plant having ornamental, ritual, medicinal, anthelmintic, insecticidal, colorant, food and forage applications.[17] – [18] The plant and its different parts are known to possess biological activities, viz., antiseptic, blood purifying, fly repellent, hepatoprotective, insecticidal, nematicidal and antidermatologic activities. Recently, the methanol extract of the whole plant and its essential oil were claimed to be the possible source of antifungal agents [19].

The present study is designed to evaluate the antifungal properties of *Tagetes* spp. (marigold) against destructive soil borne plant pathogenic fungus *Fusarium* and also the percentage inhibition is compared with *Azadirachta indica* (Neem- a well-known antiba-cterial and antifungal agent).

2. Material and Methods

2.1 Plant materials

Fresh leaves of *Azadirachta indica* and a number of whole plants and plant parts as well of *Tagetes* were collected from local area of Patna. The plants were authenticated by taxonomist from Botany Department, P.U., Patna.

2.2 Extract preparation

Collected fresh leaves, whole plants and plant parts of the aforesaid plants were washed separately with tap water followed by sterile distilled water and dried in shade. The dried materials were finally grinded to powder, sealed in polythene bags and stored away from light and moisture until used for extraction [20] – [22].

2.3 Aqueous extract - 50gm of each materials were soaked in 200ml of distilled water for 30 minutes and then boiled to half volume. After cooling, filtered with muslin cloth followed by Whatman filter paper no.-1 and kept in dark glass bottles at 5 °C in a refrigerator.

2.4 Ethanolic extract - 50gm of each materials were separately homogenized in 200ml mixture of ethanol and distilled water (50:50, v:v) and left in dark bottles for 72hrs on shaker. Then extracts were filtered with muslin cloth, followed by Whatman filter paper no.-1, in other dark bottles and exposed to 60 °C in water bath for 30 minutes for ethanol evaporation. Extracts were stored in dark bottles at 5°C.

2.5 Isolation of pathogens

Fusarium species were isolated through serial dilution culture technique of soil collected from vegetable crop fields. PDA (Potato Dextrose Agar) was used as growth medium. Further a pure culture of each colony type, growing in petri plates, was obtained and maintained by sub-culturing. The temperature was maintained at 25 ± 2 °C. Cultures were identified on the basis of macro and microscopic characteristics, reverse surface coloration of colonies, conidial morphology and slide culture technique [23] – [27]. The technique of slide culture was also used to identify the species of *Fusarium* [28] which allow the direct microscopic observation of morphological structure of taxonomic value.

Identification of *Fusarium* species as *Fusarium solani* and *Fusarium moniliforme* was also confirmed by IARI, New Delhi.

Fig 1:- Different fungal-colonies growing in PDA (serial dilution culture of soil).

Fig 2:- Isolation of *Fusarium* species from petri plates of soil culture.

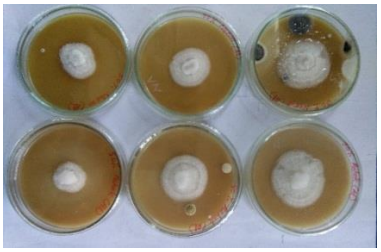
2.6 Antifungal screening

Different concentration viz-10%, 20%, 30%, 40% and 50% of extracts (both aqueous and ethanolic) of aforesaid plants were screened for their antifungal activities through poisoned food technique [29]. Proportionate amount of extracts of two botanicals were added to separate flasks containing media to prepare media having 10% -50% concentration of extracts. To avoid bacterial contamination an antibiotic (Chloramphenicol, 0.10 mg/l) was supplemented to the media. 20 ml of media was poured into petri plates, allowed to solidify and inoculated individually with 5mm diameter discs of the tested *F. solani* and *F. moniliforme*. Plates with media, not supplemented with extracts and inoculated with *Fusarium* species, served as negative control. After seven days of incubation at 25 ± 2°C, orthogonal measurements of colonies were taken using the control plates as a reference. The percentage inhibition of growth was calculated according to following formula-

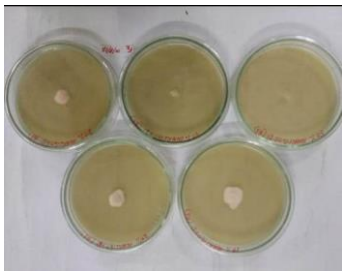
% inhibition = (dc-dt /dc) 100

Where, dc = Average increase in mycelial growth in control.

dt = Average increase in mycelial growth in treatment [30].



A



B

Fig 3:- Comparative study of the inhibitory effect of extracts of *A. indica (A)* and *Tagetes erecta (B)* on *Fusarium solani.*

Fig 4a

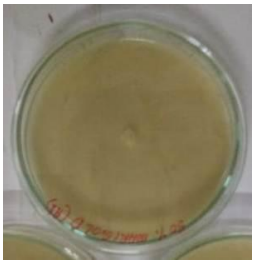


Fig 4b

Fig 4:- Growth of *Fusarium* in 50% alcoholic extract of *A. indica* (4a) and *Tagetes erecta* (4b).

3. Results and Discussion

Results presented in Table 1, 2, 3 and 4 show the inhibitory effect of aqueous (Table-1 and 3) and ethanolic (Table-2 and 4) extracts of the two botanicals against *Fusarium* species. Effects of the extracts of the two botanicals were shown in Table 1 and 2 on *Fusarium solani* and in Table 3 and 4 on *Fusarium moniliforme*.

Table -1 Inhibitory effect of aqueous extracts of *Azadirachta indica* and *Tagetes erecta* on mycelial growth of *Fusarim solani.*

	Azadirachta indica		*Tagetes erecta*	
Different concetrat-ion of aqueous extracts	Mycelial growth in mm	% Inhibition	Mycelial growth in mm	% Inhibition
10%	82.5	8.33	80.25	10.83
20%	77.25	14.16	73	18.89
30%	66.5	26.11	61.5	31.67
40%	56	37.77	42	53.33
50%	44	51.11	34	62.22

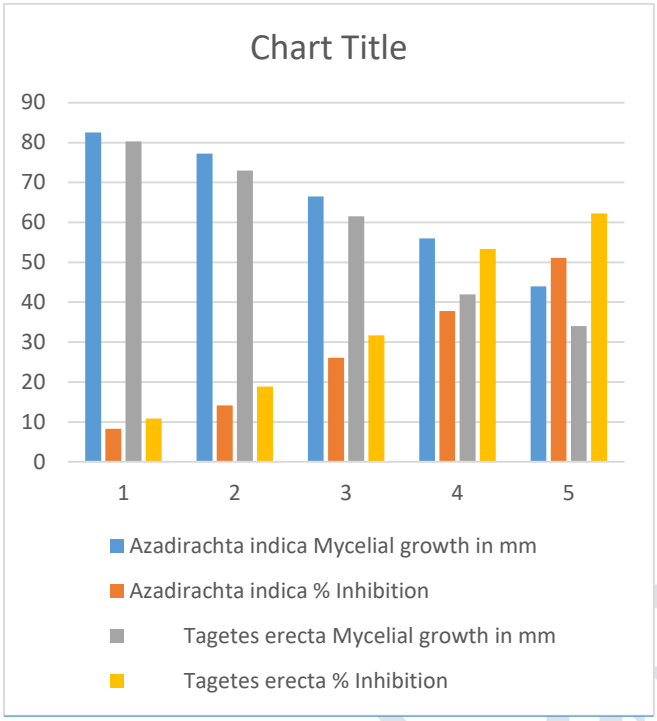


Fig 5:- Graphical representation of Table-1

Table -2 Inhibitory effect of ethanolic extracts of *Azadirachta indica* and *Tagetes erecta* on mycelial growth of *Fusarim solani.*

	Azadirachta indca		*Tagetes erecta*	
Different concentrat-ion of ethanolic extracts	Mycelial growth in mm	% Inhibition	Mycelial growth in mm	% Inhibition
10%	77.5	13.88	63	30
20%	68.0	24.44	46.5	48.33
30%	58.5	35.00	27	70
40%	37	58.88	15.33	82.97
50%	26.25	70.83	00	100

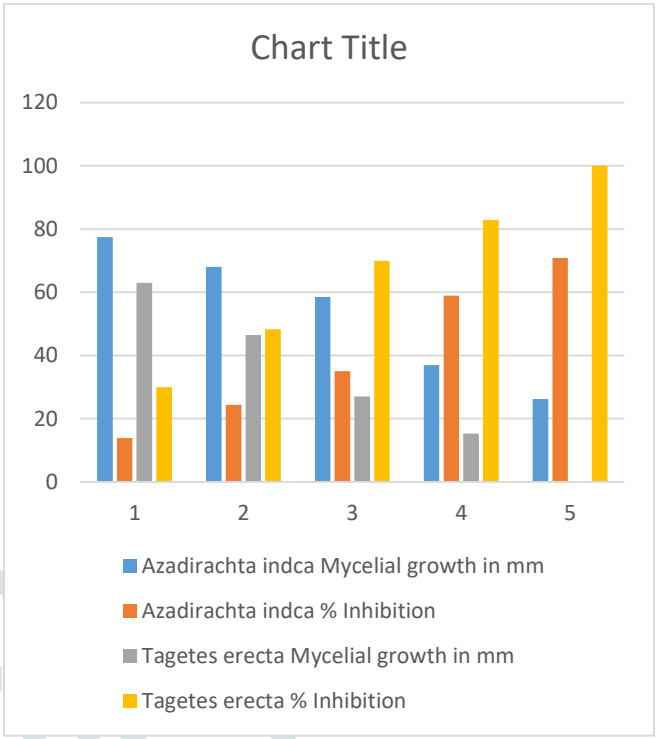


Fig 6:- Graphical representation of Table-2.

Table -3 Inhibitory effect of aqueous extracts of *Azadirachta indica* and *Tagetes erecta* on mycelial growth of *Fusarium moniliforme.*

	Azadirachta indica		*Tagetes erecta*	
Different concentrat-ion of aqueous extracts	Mycelial growth in mm	% Inhibition	Mycelial growth in mm	% Inhibition
10%	81	10	78.5	12.78
20%	73.5	18.33	70	22.22
30%	63.5	29.72	56.25	37.5
40%	50	44.44	39	56.67
50%	41	54.44	28.5	68.33

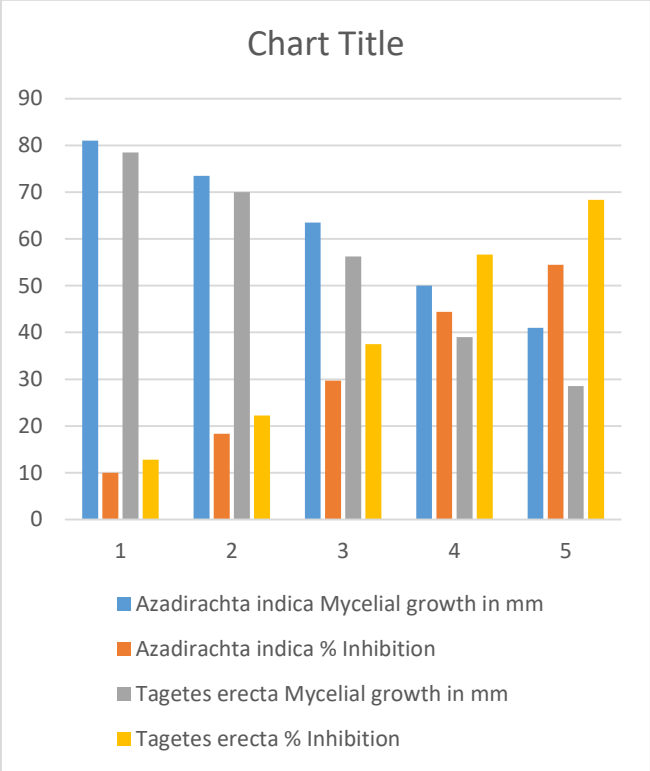


Fig 7:- Graphical representation of Table-3.

Table -4 Inhibitory effect of Ethanolic extracts of *Azadirachta indica* and *Tagetes erecta* on mycelial growth of *Fusarium moniliforme.*

	Azadirachta indica		*Tagetes erecta*	
Different concentrat-ion of ethanolic extracts	Mycelial growth in mm	% Inhibition	Mycelial growth in mm	% Inhibition
10%	70	22.2	60.5	32.78
20%	60	33.33	48	46.67
30%	48.5	46.11	37.5	58.33
40%	36	60	6.5	92.78
50%	24.5	72.77	00	100

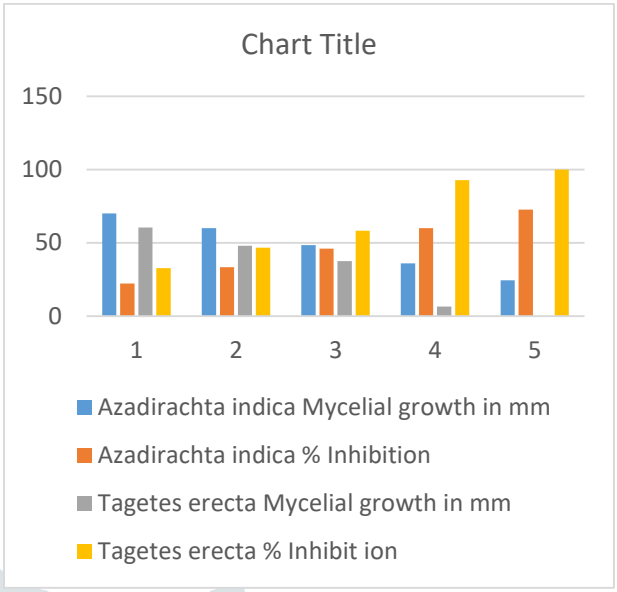


Fig 8:- Graphical representation of Table-4.

For both the plants extracts, radial growth of tested fungi decreased significantly with increased concentration of added extracts. The ethanolic extracts were found more effective than aqueous one and also *Tagetes erecta* was found more effective in both the conditions. Also the botanical control of *Fusarium moniliforme* was more than *Fusarium solani*. The most interesting observation was that, 50% ethanolic plant extract concentration of *Tagetes erecta* showed 100% inhibition of mycelial growth for both *Fusarium solani* as well as *Fusarium moniliforme*. The result could be used as an indicator to exploit *Tagetes* spp as a fungicide.

3.1 Conclusion

This study was focused on screening the antifungal activity in some common botanicals to use as natural, harmless fungicide, aiming at partial or total replacement of chemical synthetic fungicide to control soil borne diseases in vegetable crops. These results augur well for the practical use of marigold as a source of effective and easily available botanical pesticide to resource-poor farmers against various diseases of vegetable crops and may also practiced for other crops. The results also indicate that the plants are endowed with pesticidal properties that can be harnessed cheaply for use in agriculture and other related fields in an eco-friendly manner by replacing synthetic pesticides. For the conservation of biodiversity aiming to maximize food production and minimizing health hazards, botanicals may stand as the most promising source of bio-active products of plant origin.

3.2 Future Scope

The results obtained in the present study reflect light on successful use of botanicals in the development of safe and cheap compounds as pesticides which provide a potent tool to control not only *Fusarium* species but also other pathogens and getting success in the avoidance of environmental pollution and side effect of pesticides. To make their use more meaningful, economical, feasible and environmentally safe, research efforts are needed to find out the toxic compounds present in them and their mode of action.

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