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Eco-friendly management of fusarium wilt of pigeonpea *in vitro* condition

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Abstract
A study was conducted in plant pathology laboratory, SHUATS during 2019 to find out the *in vitro* efficacy of few botanicals (i.e. Neem oil, Eucalyptus oil, Castor oil), bio-agent (i.e. *Trichoderma viride)* in controlling of *Fusarium udum* causing wilt of Pigeonpea. The pathogen was isolated from the local fields of Pigeonpea and was identified by its morphological characters. Botanicals individually and in combination with the bio-agent were evaluated at 5% concentrations and to find the radial growth of the pathogen. The complete inhibition was obtained with Eucalyptus oil, Eucalyptus oil+ *Trichoderma viride* followed by *Trichoderma viride*, Neem oil+ *Trichoderma viride* and Castor oil+ *Trichoderma viride*. The present experiment was carried out by dual culture techniques and food poison techniques at same selected concentration.

Keywords: Neem oil, eucalyptus oil, castor oil, *Trichoderma viride* and *Fusarium udum*

1. Introduction
Pigeonpea (*Cajanus cajan* L.) is an important legume crop from family Fabaceae, where it is used as a major source of protein in human diet, plays important role in food security ,subsistence agriculture because of its varied usages in food, fooder, fuel, integrated farming system, soil conservation and biological nitrogen fixation (Reddy et al., 2005) [7]. It is cultivated in Australia, USA, Africa, India, Indonesia and some countries of S. America. It requires the optimum temperature for proper growth and development i.e., 18-38 ℃. Pigeonpea has a wide range of products, including the dried seed, pods and immature seeds used as green vegetables, leaves and stems, as well as from the leaf fall and recycling of the nutrients (Snapp *et al*., 2002)
Mostly attacked by *Fusarium spp*. which caused wilt disease. It shows very severe in field conditions. The pathogen is both soil and seed borne. The genus *Fusarium* have wide host range and survives for long time in the field in the absence of host plant. Wilt can be diagnosed by symptoms like loss of turgidity, slight inter-veinal chlorosis, internal browning of xylem vessels, and a purple band on stem extending upwards from the base.

2. Materials and Methods
2.1. Sample collection
The sample was collected from infected plant from local fields of pigeon pea. The infected plant parts and rhizosphere region and non- rhizosphere were plucked out in to a polythene bag and preserved for the further use.

2.2. Isolation and identification: The small pieces of infected roots of plants were cut about 2-4 mm length and sterilized with 0.5% mercuric chloride solution for 30 sec, then washed with distilled water. The section were placed in potato dextrose agar plates and incubated in room temperature for 7 days. After 7 days the mycelia of the fungus was observed and pure culture was maintained in PDA slants. The fungus was identified by its morphological characters.

2.3. Evaluation of botanicals
Total of 3 botanicals *viz*. Neem oil, Eucalyptus oil, Castor oil at 5% concentration were evaluated *in vitro* on radial growth of *Fusarium udum* applying poison food techniques (Nene and Thapliyal, 1993) [7] using Potato dextrose agar (PDA) as basal culture media.

2.3.1. Poison food technique

The principle involved in this techniques was to make the nutrient medium toxic with a fungitoxicant and allow the text fungi to grow on it and study the mycelial inhibition.100 ml of PDA was taken in 250 ml flask and the botanicals were added at 5% concentration and sterilized.

Later this PDA was poured in petriplates and inoculated with 5 mm discs of test fungal culture which were cut using cork borer.

2.3.2. Dual Culture

Dual culture technique was used to study the antagonism of *T. viride* in combination with the above botanicals at same concentrations. Discs of 5mm dia fungal mycelium for both *T. viride* and *Fusarium udum* were cut and placed in petriplates containing different botanical treated PDA. Plates with only mycelia discs of text pathogen served as control. Every treatments had 3 replications and all operations were conducted aseptic condition under laminar air flow chamber. The percent inhibition of the pathogen was calculated by the following formula.

$$\text{Percent inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

3. Result and Discussion

We were tested the dual culture method to find out the antagonistic activity against the *Fusarium udum in vitro* condition and it was observed that *T. viride* was the most effective bio-agent. Eucalyptus oil and Eucalyptus oil+ *T. viride* showed the best effect in the below table.

After 48 hrs of inoculation T_3 (Eucalyptus oil) and T_6 (Eucalyptus oil + *Trichoderma viride*) have shown least mycelia growth i.e., 0 mm, followed by T_2 Neem oil (15.42 mm), T_5 Neem oil + *Trichoderma viride* (15.78 mm) and the maximum mycelia growth was seen in T_0 control (20.75).

After 72 hrs of inoculation T_3 (Eucalyptus oil) and T_6 (Eucalyptus oil + *Trichoderma viride*) have shown least mycelial growth i.e., 0 mm, followed by Castor oil + *Trichoderma viride* (23.43), T_1 *Trichoderma viride* (24.64 mm) and the maximum mycelial growth was seen in T_0 control (32.35).

After 96 hrs of inoculation T_3 (Eucalyptus oil) and T_6 (Eucalyptus oil + *Trichoderma viride*) have shown least mycelial growth i.e 0 mm, followed by T_1 *Trichoderma viride* (25.16 mm), T_5 Neem oil + *Trichoderma viride* (25.57 mm) and the maximum mycelial growth was seen in T_0 control (41.5). After 120 hrs of inoculation T_3 (Eucalyptus oil) and T_6 (Eucalyptus oil + *Trichoderma viride*) have shown least mycelial growth i.e 0 mm, followed by T_1 *Trichoderma viride* (25.35 mm), T_5 Neem oil + *Trichoderma viride* (27 mm) and the maximum mycelial growth was seen in T_0 control (53.33).

The below table shows the maximum inhibition percentage of T_3 Eucalyptus oil (100%) and T_6 Eucalyptus oil + *T. viride* (100%) followed by T_1 *T. viride* (53.09%) , T_5 *T. viride* + Neem oil (49.34%). The minimum inhibition growth in T_0 control (0 %).

Similar findings were given by Joseph Babu *et al*. (2008) who evaluated the *in vitro* efficacy of different plant extracts viz. *Azardirachta indica, Artemessia annua, Eucalyptus globules, Ocimum sanctum* and *Rheum emodi* to control brinjal wilt pathogen. Different concentrations 5, 10, 15 and 20 % of plant extracts was used in this study. Among the different extracts 20% of *Azardirachta indica* was found most effective followed by *Rheum emodi* and *Eucalyptus globules.*

Another similar finding include Raju *et al*. (2008) who conducted a trail on three antagonists *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas fluorescenes* against *Fusarium udum in vitro. T. viride* was best in inhibiting the growth of the pathogen by 73.6%.

Table 1: Percent inhibition of *Fusarium udum* by fungal antagonists using dual culture technique under *in vitro* condition.

Treatments	48 Hrs		72 Hrs		96 Hrs		120 Hrs	
	MG	%I	MG	%I	MG	%I	MG	%I
Control	20.78	-	32.35	-	41.50	-	53.33	-
T. viride	18.78	15%	24.64	23,83%	25.35	39.03%	25.16	53.09%
Neem oil	15.42	25.60%	25.57	21.05%	34.57	16.86%	44.83	15.95%
Eucalyptus oil	0	100%	0	100%	0	100%	0	100%
Castor oil	18.71	9.66%	31.14	3.71%	41.21	0.72%	51.83	2.81%
Neem oil+ *T. viride*	15.78	24.15%	24.92	22.91%	25.57	38.55%	27	49.34%
Eucalyptus oil+ *T. viride*	0	100%	0	100%	0	100%	0	100%
Castor oil+ *T. viride*	19.28	7.24%	23.42	27.55%	26.71	35.66%	27.83	47.84%
F test	S		S		S		S	
SE.d(+)	0.774		0.659		1.460		1.563	
CD (0.05)	1.633		1.402		3.094		3.312	

*MG= Mycelial growth (in mm), **%I= Inhibition percent

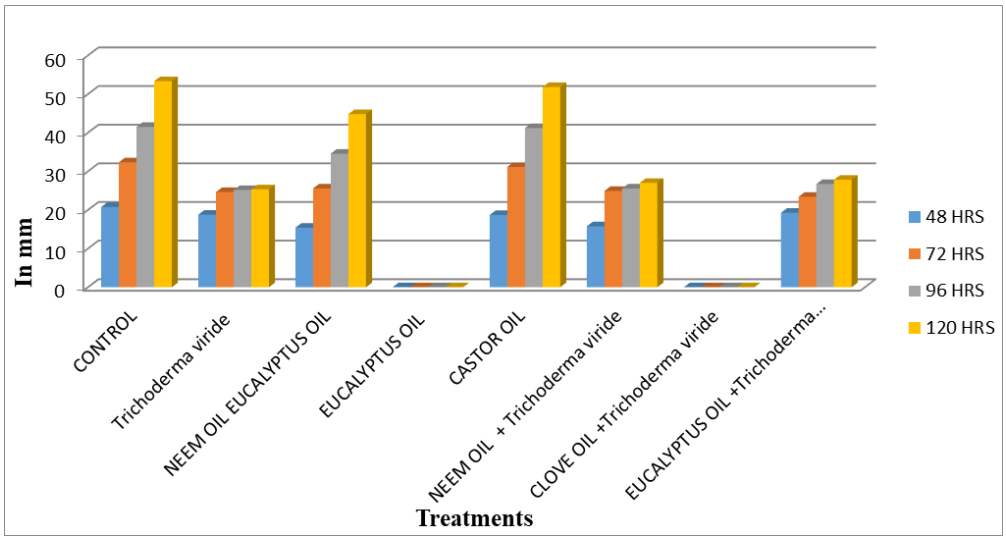

Fig 1: Mean colony diameter of *Fusarium udum*

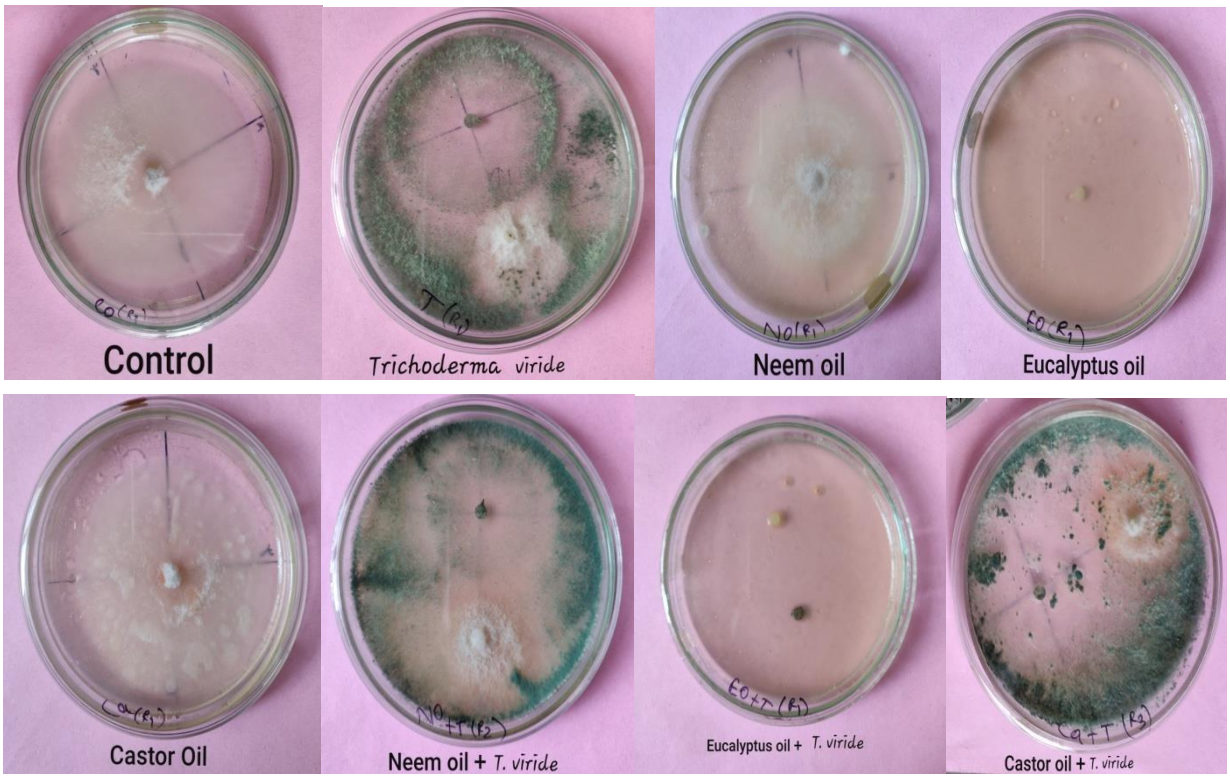

Plate 1: Effect of treatments *in vitro* conditions

Conclusion

In summary, our results revealed that the three essential oils and one bio-agent *Trichoderma viride* tested in this study could efficiently suppress conidial germination and mycelial growth of *F. udum in vitro*. The essential oil combination with Trichoderma viride was more effective than single treatment. Therefore, developing commercial products containing several essential oils might be promising as eco-friendly strategy to control Fusarium wilt.

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