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Eco-friendly Management of Stem Canker Disease of Pigeonpea caused by *Phoma cajani*

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ABSTRACT: An investigation was carried out to determine the efficacy bioagents and essential oils against *Phoma cajani* causing stem canker disease of pigeonpea. The bioagents *viz*., *Trichoderma* spp, *Bacillus* spp and *Pseudomonas* spp were found effective against test pathogen. Among them, *T. asperellum* was found most effective and recorded highest percent mycelial inhibition (72.91 %). Followed by *T. virens,* (68.85 %) and *T. hamatum* (64.59 %). The least mycelial growt hinhibition which recorded in *T. harzianum* (63.36 %). The two bacterial antagonists tested, *Bacillus subtilis* was found most effective and recorded highest percent mycelial inhibition (51.39 %) followed by *Pseudomonas fluorescens* (41.64 %) against maximum per cent mycelial growth inhibition was (100%) over untreated control. The radial per cent mycelial growth inhibition recorded with the essential oils tested was ranged from 45.74 to 100.00 %. However, it was significantly highest and per cent mycelial growth inhibition with essential oils citronella oil and lemon grass oil. These were followed by lavender oil (91.36 %), eucalyptus oil (63.55 %), neem oil (52.10 %), olive oil (47.19 %), and peppermint oil (45.74 %) with the maximum percent mycelial growth inhibition being (100 %) over the untreated control. So, organic management might be a better option to control against *Phoma cajani* causing stem canker disease of pigeonpea also having environment friendly.

Keywords: Stem Canker Disease, *Phoma cajani*, Ecofriendly management, Essential oils, Pigeonpea, Bioagent.

INTRODUCTION

Pigeon pea (*Cajanus cajan* (L) Millsp) is perennial member of Fabaceae family. It has over 350 vernacular names (In Sanskrit: Adhaki, Hindi: Arhar, English: Pigeon pea, Bengali:Tur). Pigeonpea is grown in over 25 tropical and subtropical countries, either as a stand-alone crop or in mix crop with legumes such as groundnut, soyabean, black gram and green gram. Pigeonpea, being a legume, nourishes the soil through symbiotic nitrogen fixation. Pigeon pea is a legume reported to contain 20-22% protein, 1.2 % fat, 65%carbohydrate and 3.8% ash (Anonymous, FAO 1982). These food and fodder crops also promote soil fertility by scavenging atmospheric nitrogen, adding organic matter, boosting phosphorus availability, and enhancing the physical, chemical, and biological properties of the soil. As a result, they are critical components of a wide range of cereal-based cropping systems on marginal and submarginal soils, where they support intensive agriculture and agricultural practises employed by small and marginal farmers.

More than 100 diseases, including fungus, bacteria, viruses, mycoplasma, and nematodes, may infect pigeonpea, which is a severe biological limitation on productivity (Nene *et al.,* 1989). Fortunately, only a few of these result in financial losses. While some of the diseases, like stem canker and fusarium wilt, are common throughout the world, others, like sterility mosaic and witches' broom, are region-specific.

Pigeonpea Phoma stem canker, which is caused by *Phoma cajani* is sporadic but can occasionally become extremely damaging and pandemic in favourable environments. When the crop is in the flowering stage from November to February, it becomes more serious in dry weather and at low temperatures. It was a minor disease when it was first discovered by Khune and Kapoor (1981) in India. But it has become increasingly serious in recent years, inflicting greater economic losses to the regions of Maharashtra, Madhya Pradesh, Chattisgad, and Andhra Pradesh that produce pigeonpeas. During the periodic surveys and critical inspections, the disease was frequently observed in farmers' fields and experimental plots, where it caused 5 to 50 percent of plant death in mature plants (Behera *et al.,* 2017).

The pathogen is mostly soil-borne and has ability to survive saprophytically on agricultural debris. Due to the high cost, danger, and disruption of the biological balance caused by the necessary large-scale soil application of chemicals, chemical treatment of the disease is consequently difficult, impracticable, and uneconomical (Songa *et al.,* 1991). Therefore, by using alternative techniques, efforts must be made to reduce pathogen activity and limit losses below economic threshold levels. Biocontrol techniques include the

addition of mycoparasites like Trichoderma or the manipulation of hostile rhizosphere microorganisms and use of essential oils is important.

MATERIAL AND METHODS

The present experiment was conducted at Department of Plant Pathology, Post Graduate institute, Dr. PDKV Akola (M.S.) during 2021-22. The four fungi and two bacterial bioagents was evaluated *in vitro* against *P. cajani,* by applying Dual Culture Technique (Dennis and Webster 1971). The essential oils were evaluated *in vitro* for their antifungal activities against *Phoma cajani*, requisite quantity of each essential oils on the basis of active ingredient (a.i) was calculated and Tween (for oil dispersion) was thoroughly mix with autoclaved and cooled (40-45ºC) PDA in conical flasks to obtain desired concentration of 0.5 %.

A. Collection and isolation of Phoma isolates

Phoma isolates were collected from major pigeonpea growing area from different agro climatic zones of Indian. Isolations were made by cutting infected parts from the junctions of healthy and diseased leaf regions and surface sterilising with 70% ethanol. Sterilised bits were transferring it to a petri plate containing sterilised PDA (Potato Dextrose Agar) media. The cultures of *Phoma* isolates were maintained on PDA (Hi-Media, Mumbai) slants stored at 4ºC for further study.

B. Purification of Phoma cajani

The fungus was further purified by single hyphal tip method. They are grown by inoculating in the centre of a plain agar plate. The fungus spreads out with its hyphal strands in search of nutrients. These hyphal strands could be located under low power of the microscope, and the isolated hyphal tips marked. These tips were carefully transferred to potato dextrose agar slants to obtain the pure cultures of *Phoma cajani*. The culture was maintained by sub-culturing on potato dextrose agar medium at room temperature.

C. Pathogenicity test

Pathogenicity of *Phoma cajani* in pigeonpea was proved by applying spray inoculation with spore and mycelial suspension of the pure culture of *Phoma cajani* on the seedling of susceptible cultivar of Maruti in earthen pots. *Phoma cajani* spore suspension of 10^4µL spores was taken from the 10-day- old culture and inoculated on susceptible cultivar of Maruti in earthen pots. A control was separately maintained in which all the operations were similar except the addition of the fungal culture. Symptom appearance was observed at regular intervals. Koch's postulates were confirmed by reisolating the fungus from diseased stem and compared with the original test fungus.

The PDA medium amended with essential oils were poured separately @ 20ml per petri plate. After solidification of poisoned medium, the plates were inoculated with 0.5mm mycelium disc of *P. cajani* obtained from seven days old culture of pathogen. Plates containing um-amended medium served as control. The inoculated plates were incubated in B.O.D. incubator at 26±2˚C. The colony diameter of culture was recorded when plates under control were fully covered. The efficacy of bioagents and essential oils was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the following formula (Vincent, 1927).

$$\text{Per cent Growth} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in treated plate}}{\text{Inhibition colony growth in control plate}} \times 100$$

RESULTS AND DISCUSSION

A. In vitro Evaluation of Bioagents

Chemicals are dramatic, attractive, rapid, and convincing, even to an unqualified farmer, yet there is growing global concern over environmental degradation as a result of the increased use of toxic pesticides, especially fungicides. A wide range of microbes have been implicated as plant pathogen biocontrol agents, sometimes with compelling evidence. As a result, research was carried out to identify an effective biocontrol agent against *Phoma cajani* and to establish a biocontrol technique as a viable component.

The results obtained on mycelial growth and inhibition of *P. cajani* with four fungal antagonists *viz. T. asperellum, T. virens, T. hamatum* and *T. harzianum* and two bacterial antagonists *viz. Bacillus subtilis* and *Pseudomonas fluorescens* are presented in Table 1, Fig. 1 and Plate 1 revealed that, all the bioagents evaluated exhibited fungistatic/antifungal activity against *P. cajani* and significantly inhibited it's growth.

Average radial mycelial growth. The four fungal antagonists were tested, among them *T. asperellum* was found most effective and recorded least linear mycelial growth (24.38 mm). Followed by *T. virens,* (28.04 mm) and *T. harzianum* (31.87 mm), whereas, highest mycelial growth recorded in *T. hamatum* (32.97 mm). The two bacterial antagonists were tested, among them *Bacillus subtilis* was found most effective and recorded least linear mycelial growth (43.75 mm). followed by *Pseudomonas fluorescens* (52.53 mm) compared to the untreated control maximum mycelial growth (90.00 mm).

Average mycelial growth inhibition. The four fungal antagonists tested, *T. asperellum* was found most effective and recorded highest percent mycelial inhibition (72.91 %). Followed by *T. virens,* (68.85 %) and *T. hamatum* (64.59 %). The least mycelial growth inhibition which recorded in *T. harzianum* (63.36 %). The two bacterial antagonists tested, *Bacillus subtilis* was found most effective and recorded highest percent mycelial inhibition (51.39 %) Followed by *Pseudomonas fluorescens* (41.64 %) against maximum per cent mycelial growth inhibition was (100%) over untreated control.

These results were in conformity to the finding of several earlier worker. *T. asperellum* was found most

effective and recorded highest percent mycelial inhibition Muthulakshmi and Parthasarathy (2019); Bharsakal *et al.* (2015); Mamata *et al.* (2022).

Table 1: *In vitro* bio efficacy of different bioagents against *P. cajani.*

Tr. No.	Bioagents	Average Colony Diameter(mm)	Average % Inhibition
1.	*Trichoderma asperellum*	24.38	72.91
2.	*T. harzianum*	31.87	64.59
3.	*T. hamatum*	32.97	63.36
4.	*T. virens*	28.04	68.85
5.	*Bacillus subtilis*	43.75	51.39
6.	*Pseudomonas fluorescens*	52.53	41.64
7.	Control (untreated)	90.00	0.00
	SE±	1.16385	-
	CD at 0.01%	4.51057	-

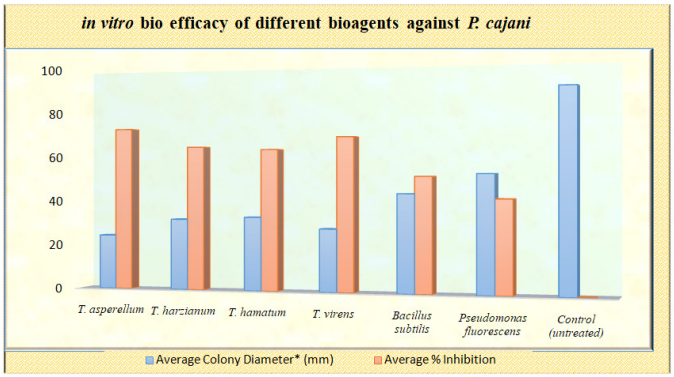

Fig. 1. *In vitro* bio efficacy of different bioagents against *P. cajani.*

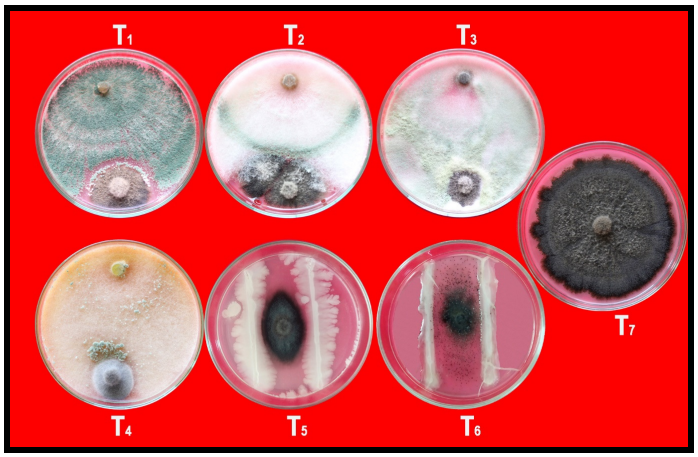

Plate 1. *In vitro* bio efficacy of different bioagents against *P. cajani.*

B. In vitro evaluation of essential oils

Seven essential oils *viz*., eucalyptus oil, lemon grass oil, neem oil citronella oil, levender oil, olive oil and peppermint oil at 0.5 %were evaluated against the *P. cajani* as described in material and methods section. The data on the per cent inhibition of radial growth of the test pathogen is presented in Table 2.

Average radial mycelial growth. The results (Table 2 Fig. 2 and Plate 2) revealed that the seven essential oils (each at 0.5 % concentration) tested against isolates exhibited a wide range of *P. cajani* radial mycelial growth. The average radial mycelial growth measured with the essential oils tested ranged from 0.00 to 48.83 mm when compared to the untreated control. Citronella oil and lemon grass oil completely inhibited the radial mycelial growth/colony diameter of all *P. cajani* isolates. Followed by lavender oil (7.78 mm) and eucalyptus oil (32.81 mm). The maximum mycelium growth was found in neem oil (43.11 mm), olive oil (47.53 mm) and peppermint oil (48.83 mm), compared to the maximum mycelial growth (90.00 mm) in the untreated control.

Average mycelial growth inhibition. Average radial per cent mycelial growth inhibition recorded with the essential oils tested was ranged from 45.74 to 100.00 %. However, it was significantly highest and per cent

mycelial growth inhibition with essential oils citronella oil and lemon grass oil. These were followed by lavender oil (91.36 %), eucalyptus oil (63.55 %), neem oil (52.10 %), olive oil (47.19 %), and peppermint oil (45.74 %) with the maximum percent mycelial growth inhibition being (100 %) over the untreated control.

Table 2: *In vitro* efficacies of different essential oils against *P. cajani.*

Tr. No.	Essential oils	Average Colony Diameter* (mm)	Average % Inhibition
1.	Eucalyptus oil	32.81	63.55
2.	Lemon grass oil	0.00	100.00
3.	Neem oil	43.11	52.10
4.	Citronella oil	0.00	100.00
5.	Lavender oil	7.78	91.36
6.	Olive oil	47.53	47.19
7.	Peppermint oil	48.83	45.74
8.	Control	90.00	0.00
	SE±	1.48956	-
	CD at 1%	5.55002	-

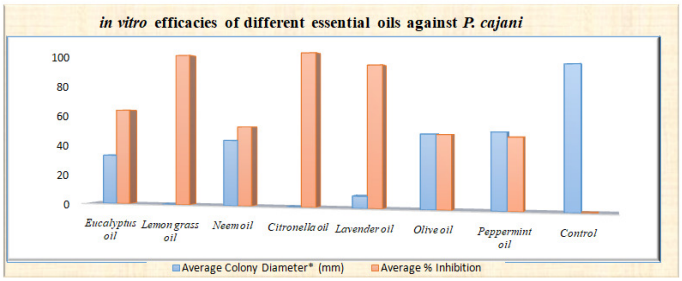

Fig. 2. *In vitro* efficacies of different essential oils against *P. cajani.*

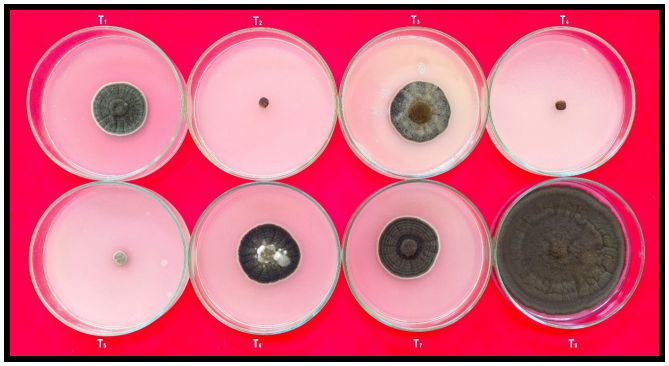

Plate 2. *In vitro* efficacies of different essential oils against *P. cajani.*

CONCLUSIONS

In present investigation the bioagents *viz.*, *T. asperellum* was found most effective and recorded highest percent mycelial inhibition (72.91 %). However, in essential oils, cent per cent mycelial growth inhibition with citronella oil and lemon grass oil. These were followed by lavender oil (91.36 %), eucalyptus oil (63.55 %), neem oil (52.10 %), olive oil (47.19 %), and peppermint oil (45.74 %) with the maximum percent mycelial growth inhibition being (100 %) over the untreated control. So, organic management might be a better option to control against *Phoma cajani* causing stem canker disease of pigeonpea.

FUTURE SCOPE

(i) Surveillance of the pathogen has to be undertaken to observe the rhythmic changes in disease.
(ii) Field management of *Phoma* sp., infecting pigeonpea through integrated approach.

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Conflict of Interest. None.

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