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Efficacy of Botanical Extracts and Organic Amendments against *Sclerotium rolfsii* Sacc. Incitant of Collar Rot of Chickpea and its Compatibility with Potential *Trichoderma* Isolate

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ABSTRACT

Collar rot of Chickpea caused by *Sclerotium rolfsii* Sacc. is an economically important soil borne disease. Isolation of pathogen was carried out by tissue segment method from infected collar region of Chickpea. A total of 4 leaf extracts and 5 organic amendments were evaluated against *Sclerotium rolfsii* at 5%, 10% and 15% under *in vitro* condition through poisoned food technique. The effective leaf extract and organic amendment obtained from poison food technique and potential rhizosphere antagonist *Trichoderma* isolate were subjected to check the compatibility. Percentage mycelial inhibit ion was calculated. All leaf extracts and organic amendments at 15% concentration were found to be significantly superior to 5% and 10%. Average Percent mycelial inhibition was recorded highest with Neem leaf extract (29.81%) and Pongamia leaf extract observed with least average inhibition (19.81%). Groundnut cake showed the lowest average percent of mycelial inhibition (15.37%) of the five organic amendments studied, while vermicompost showed the highest average percent of mycelial inhibition (50.19 %).The number of sclerotia per plate was found to be the lowest among the leaf extracts tested, with neem leaf extract at 15%. Among different organic amendments tested, number of sclerotia per plate was found minimum with vermicompost. The potential *Trichoderma* isolate showed 100% compatibility with neem leaf extracts in all concentrations whereas, Vermicompost extract showed 6.67% inhibition at 15% and showed 100% compatibility with 5 and 10% concentration.

Keywords Chickpea, Collar rot, Leaf extracts, Organic amendments.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a major pulse crop grown and consumed worldwide. Chickpea is rich in proteins, ranging from 20 to 25% depending on variety and environmental conditions. It comprises 60-65 % carbohydrates, 6 % fat, and is enriched with

minerals and vitamins (Jukanti *et al.* 2012, Wallace *et al.* 2016). Diseases of chickpea considered as the major constraint for improvement of the crop yield. Three soil borne diseases such as wilt, collar rot and dry root rot are considered economically important. Among these, collar rot caused by *Sclerotium rolfsii* has an important role which about 10–30% yield loss has been observed annually (Maurya *et al.* 2008). *S. rolfsii* is a soil-borne plant pathogenic fungus with a host range of about 500 plant species and 100 families, the majority of which are dicotyledonous plants (Eslami *et al.* 2015, Billah 2017). The disease symptoms mainly occur in wet soil conditions, which appear within two weeks of sowing and the foliage turns yellow before drying and the death of the plant. The collar region shows the rotting symptom and the rotted area covers with white mycelial strands of *S. rolfsii* with mustard like sclerotia around the infected portion of root (Lahre 2008, Khan *et al.* 2020).

Management of soil-borne pathogens is difficult only with chemicals due to the widespread host range, abundant growth of the pathogen and its capability of producing sclerotia, which has longer persistence in the soil (Sennoi *et al*. 2013). Therefore, the use of biological control methods is effective for long term management of soil borne diseases and its efficiency is highly dependent on the integrated approaches to maintaining soil health and controlling soil borne pathogens. *Trichoderma* species are known to suppress infection of root by soil borne pathogens (Rasu *et al.* 2012, Mayo *et al.* 2015). *Trichoderma* strains exert biocontrol against fungal phytopathogens through mycoparasitism, competition, and antibiosis (Benitez *et al.* 2004, Mukhopadhyay and Kumar 2020). Plant extracts and organic amendments may be used as an alternative source for controlling soil borne diseases. The application of organic amendments suppresses a wide range of soilborne pathogens (Thakkar *et al.* 2018, Vineela *et al.* 2020) by inducing physico-chemical and biological changes in soils (Zhao *et al.* 2009, Scotti *et al*. 2015, Mercado Blanco *et al.* 2018, Vida *et al.* 2020). Botanical extracts may be used as a good alternative source for controlling soil-borne diseases (Khan *et al.* 2020). The bioagents must be compatible with botanicals and organic amendments in order to be used in an integrated disease management program.

The present study was carried out to investigate the inhibitory effects of leaf extracts and organic amendments on the mycelial growth and sclerotia production of *S. rolfsii* under and its compatibility with potential antagonistic *Trichoderma* isolate *in vitro* conditions.

MATERIALS AND METHODS

Isolation of the pathogen from infected plant samples

The present study was conducted at pathology laboratory of SV Agricultural College, Tirupati. Tissue segment method (Rangaswami and Mahadevan 1999) was followed for isolation of pathogen. Infected collar portion along with some healthy portions made cut with the help of razor blade under aseptic conditions. Such small bits were surface sterilized with 1.0% sodium hypochlorite solution for one minute followed by washing with sterile distilled water three times. These pieces were transferred to sterile blotting paper to remove water adhered to sample and then aseptically transferred to petriplates containing the sterilized Potato Dextrose Agar (PDA) medium. The plates were incubated in incubator at 28±2°C and observed periodically for growth of the fungus. After attaining fungal growth, small disc measuring 5 mm was cut and transferred aseptically to the PDA slants to obtain the pure culture of the fungus.

Preparation of aqueous extracts of leaf and organic amendments

The leaf extracts such as Neem (*Azadirachta indica*), Tulsi (*Ocimum basilicum*), Mint (*Mentha* spp.) and Karanj (*Pongamia pinnata*) and five different organic amendment extracts (such as Neem cake, Cotton cake, FYM, Vermicompost and Groundnut cake) were prepared at different concentrations i.e., 5%, 10% and 15%. Fresh leaves (20 g) of different plant species were collected and macerated with 20 ml of distilled water. The sap thus extracted was first passed through four layers of muslin cloth and then filtered through two layer of muslin cloth and autoclaved at 15 psi for 20 minutes and kept in UV light for one hour.

For the preparation of aqueous extracts of or-

ganic amendments, 100 g of organic amendment in each was taken and made into powder. It was soaked in sterile distilled water at 1 g in 1.25 ml of sterile distilled water and allowed to stand overnight. The material was again grounded using a pestle and mortar and filtered through two layer of muslin cloth and the filtrate was centrifuged at 10,000 rpm for 15 minutes. The supernatant served as the standard organic amendment extract solution (100%) (Dubey 2002). The extracts of organic amendment were autoclaved at 15 psi for 20 minutes before adding to the medium.

In-vitro evaluation of leaf extracts and organic amendments against *Sclerotium rolfsii*

Antifungal activity of four leaf extracts and five organic amendment were studied under *in vitro* condition was carried out through poisoned food technique (Nene and Thapliyal 1993). Requisite quantity of extract was incorporated aseptically and individually in molten PDA to make required concentration. To avoid bacterial contamination, a pinch of Streptomycin sulphate was added at the time of pouring of media in petriplate. About 20 ml media was poured in a sterilized petriplate and allowed to solidify. A five mm disc from four days old culture of test fungus was placed in the center of medium. Medium without extract was used as control. Three replications were maintained in each treatment along with a control. The inoculated petriplates were than incubated in the BOD incubator at 28±2^{0}C. Observations were recorded for mycelial growth regularly after inoculation and sclerotia were counted 15 days after inoculation (Thakkar *et al.* 2018). Per cent inhibition of mycelial growth of pathogen was calculated by using the following formula.

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

I=% inhibition
C=Diameter of colony of control
T=Diameter of colony in treatment

Compatibility of potential *Trichoderma* isolate with effective leaf extract and organic amendment

The effective leaf extract and organic amendment obtained from poison food technique (Nene and Thapliyal 1993) and potential rhizosphere antagonist *Trichoderma* isolate obtained from our previous lab studies were subjected to check the compatibility among those and was evaluated by poison food technique.

RESULTS AND DISCUSSION

In-vitro evaluation of leaf extracts and organic amendments against *Sclerotium rolfsii*

The effect of leaf extracts and organic amendment

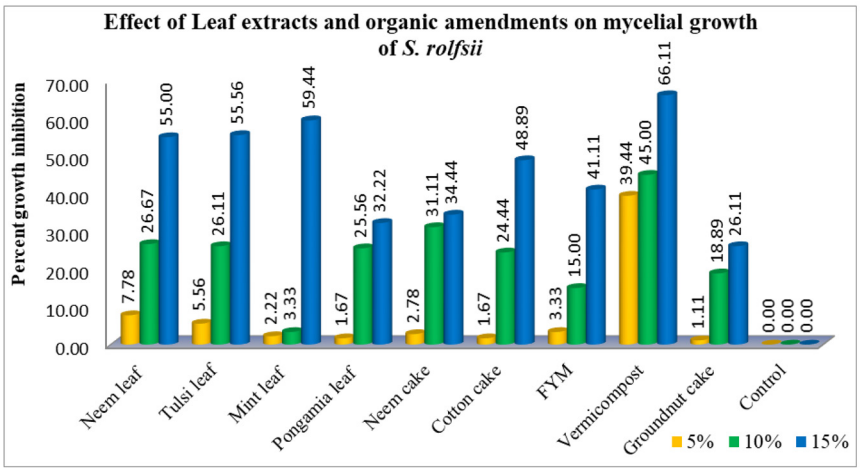

Fig. 1. *In vitro* efficacy of different leaf extracts and organic amendments against *S. rolfsii.*

Table 1. *In vitro* efficacy of different leaf extracts and organic amendments against *S. rolfsii.*

Treatment	Concentration (%)	Mycelial growth (cm)	Percent inhibition (%)	Average percent inhibition (%)	No. sclerotia per plate
Neem leaf	5	8.30	7.78^{l} (16.19)	29.81	41
extract	10	6.60	26.67^{i} (31.08)		34
	15	4.05	55.00^{c} (47.85)		15
Tulsi leaf	5	8.50	5.56^{lm} (13.56)	29.07	65
extract	10	6.65	26.11^{i} (30.71)		54
	15	4.00	55.56^{c} (48.17)		48
Mint leaf	5	8.80	2.22^{no} (8.56)	21.67	80
extract	10	8.70	3.33^{mn} (10.36)		64
	15	3.65	59.44^{b} (50.42)		53
Pongamia leaf	5	8.85	1.67^{no} (7.30)	19.81	62
extract	10	6.70	25.56^{i} (30.34)		35
	15	6.10	32.22^{gh} (34.56)		28
Neem cake	5	8.75	2.78^{o} (9.53)	22.78	91
	10	6.20	31.11^{h} (33.87)		86
	15	5.90	34.44^{g} (35.92)		65
Cotton cake	5	8.85	1.67^{no} (7.30)	25.00	73
	10	6.80	24.44^{i} (29.61)		52
	15	4.60	48.89^{d} (44.34)		38
FYM	5	8.70	3.33^{mn} (10.36)	19.81	84
	10	7.65	15.00^{k} (22.77)		61
	15	5.30	41.11^{f} (39.86)		40
Vermicompost	5	5.45	39.44^{f} (38.89)	50.19	59
	10	4.95	45.00^{e} (42.11)		44
	15	3.05	66.11^{a} (54.38)		36
Groundnut	5	8.90	1.11^{no} (6.04)	15.37	97
cake	10	7.30	18.89^{j} (25.74)		86
	15	6.65	26.11^{i} (30.71)		74
Control	-	9.00	0.00^{o} (0.00)	0.00	124
		CD	2.332		
		SE (m)	0.801		
		SE (d)	1.132		
		CV	4.168		

*Figures in parenthesis are angular transformed values,
The figures with similar alphabet do not differ significant, DAI – Days after inoculation.

on mycelial growth of *S. rolfsii* was significant. The percent of mycelial inhibition was increased as the concentration of leaf extract and organic amendment increased and the results are presented in Table1, Fig. 1 and Plate 1.

Among 4 different leaf extracts evaluated, average Percent mycelial inhibition was recorded highest with Neem leaf extract (29.81), followed by Tulsi leaf extract (29.07%) and Mint leaf extract (21.67%) while, Pongamia leaf extract observed with least average inhibition (19.81%). Among 5 different organic amendments tested, average Percent mycelial inhibition was recorded maximum with Vermicompost (50.19%) followed by Cotton cake (25%), Neem cake (22.78%), FYM (19.81%) and Groundnut cake (15.37%).

Among the three concentrations tested, 15% concentration of all leaf extracts and organic amendments was found to be significantly superior to 5% and 10%. At 15% concentration of leaf extracts, maximum of 59.44% inhibition of mycelial growth was recorded in Mint leaf extract followed by Tulsi leaf extract (55.56%), Neem leaf extract (55%) and Pongamia leaf extract (32.22%). At 15% concentration of or-

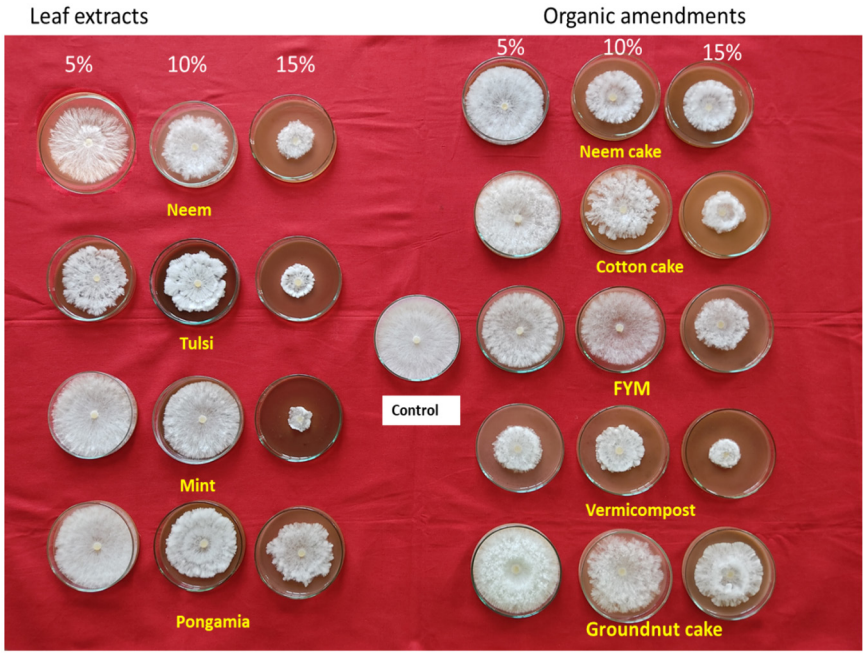

Plate 1. Effects of leaf extracts and organic amendments at different concentrations on mycelial growth of *Sclerotium rolfsii* after 4 days of incubation at 28 ± 2 °C.

ganic amendments, maximum of 66.11% inhibition of mycelial growth was recorded in Vermicompost followed by Cotton cake (48.89%), FYM (41.11%), Neem cake (34.44%) and Groundnut cake (26.11%).

Generally, the number of sclerotia per plate was decreased as the concentration of leaf extract and organic amendment increased. Among different leaf extracts tested, number of sclerotia per plate was found minimum with neem leaf extract at 15% (15). The sclerotia production was recorded maximum with Mint leaf extract (80, 64, 53) followed by Tulsi leaf extract (65,54, 48), Pongamia leaf extract (62, 35, 28) and Neem leaf extract (41,34,15) at 5%, 10% and 15% respectively. Among different organic amendments tested, maximum sclerotia production was observed with Groundnut cake (97, 86, 74), Neem cake (91, 86, 65), FYM (84, 61, 40) and Cotton cake (73, 52, 38) at 5%, 10% and 15% respectively. Number of sclerotia per plate was found minimum with Vermicompost; 59, 44 and 36 at 5%, 10% and 15% respectively.

The present findings are comparable with Farooq *et al.* (2010) who evaluated different plant extracts on mycelial growth of *Sclerotium rolfsii* and the maximum inhibition was recorded by *Azadirachta indica* (73.8%). Singh *et al.* (2012) observed that neem extract (*Azadirachta indica*) caused the inhibition of mycelial growth and sclerotial production. Suryawanshi *et al.* (2015) tested different botanicals at 10 and 20% and recorded highest average mycelial growth inhibition with Azadirachta indica (71.17%). Mohanty *et al.* (2016) reported the efficacy of different botanical extracts *S. rolfsii* at 20% concentration and considerably high inhibition of mycellial growth was noted with leaf extract of neem (*Azadirachta indica*) and patal gaurad (*Rowlphia serpentine*).

Neem plants parts shows antimicrobial role through inhibitory effect on microbial growth by potentiality of cell wall breakdown. Quercetin and beta-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties (Govindachari *et al.* 1998). *Mentha* spp. contains constituents such as menthol, menthyl acetate, linalool, and eugenol,

which alter fungal cell permeability and cause plasmolysis and cell death (Devi *et al*. 2010). This study also found that mycelium hyphae *S.rolfsii* disintegrating when treated with mint leaf extract had potential action as a biological control and therapeutic effect against disease. Moreover, at 15% concentration, the maximum percent inhibition of mycelial growth was recorded in mint leaf extract. But the number of sclerotia produced is less with neem leaf extract. In perspective of long-term management, neem leaf extract was choosen as being more effective than the others.

Nandeesha and Ravindra (2021) evaluated plant extracts at different concentrations and results revealed that 15% concentration of all plant extracts were superior to 5 and 10% and maximum mycelial growth inhibition (62.19%) was recorded in tulsi leaf extract followed by marigold leaf extract (57.11%). The extracts of many plants possess active constituents including attributed phytochemicals and alkaloids (Sahana *et al.* 2017) which have either direct antimicrobial activity (Amadioha 2003) or induce host defense response thereby resulting in reduction of disease development (Schneider and Ullrich 1994). The present findings on effect of botanical extracts are also comparable with Obongoya *et al.* (2010), Gupta *et al.* (2012), Islam and Faruq (2012), Khan *et al.* (2020).

The efficacy of organic amendment extracts reported by Senjaliya and Nathawat (2015) who evaluated 6 different organic extracts (mustard, groundnut, neem, castor, cotton cakes and FYM) and results revealed that all the extracts significantly inhibited the growth of *Sclerotium rolfsii in vitro* except FYM. Anitha *et al.* (2019) evaluated the *in vitro* efficacy of nine organic amendments against *Sclerotium rolfsii* recorded the maximum inhibition (80.11%) with mahua oil cake at 10% concentration on the mycelial growth and Vermicompost extract was found to be the least effective with 30.22% growth reduction over control.

However in the present study, Vermicompost was found most effective among different organic amendment extracts tested with 66.11% inhibition of mycelial growth at 15% concentration. This mycelium growth inhibition in the sterilized compost extract may be due to some chemical compounds elaborated during composting process and remained after the sterilization step or some thermostable extracellular metabolites. The results are also supported by You *et al.* (2019) who reported that ergosterol peroxide is a bioactive metabolite derived from powdered bamboo vermicompost that inhibits *R. solani* mycelium growth and antifungal compounds in the vermicompost are released by microbes.

Vineela *et al.* (2020) tested organic extracts *in vitro* against *S. rolfsii* at 10% concentration and the results showed that well decomposed FYM and Groundnut cake inhibited mycelial growth of *S. rolfsii* by 100% followed by Mustard oil cake (88.8%), Neem cake (60.70%), Karanja (60.7%) and Vermicompost (59.96%) which were significantly higher than control. The effect of organic amendments against *S. rolfsii* also reported by different authors including Johnson *et al.* (2003), Pawar *et al.* (2014).

Compatibility of potential *Trichoderma* isolate with effective leaf extract and organic amendment

The compatibility of potential *Trichoderma isolate* was carried out with effective leaf extract (Neem leaf) and organic amendment (Vermicompost) at 5%, 10% and 15% by poisoned food technique. Neem leaf extracts did not affect the mycelial growth of *Trichoderma* and showed 100% compatibility with

Table 2. Compatibility of potential *Trichoderma* isolate with effective leaf extract and organic amendment.

Treatment	Concentration (%)	Mycelial growth (cm)	Percent inhibition (%)
Neem leaf	5	9	0.00
extract	10	9	0.00
	15	9	0.00
Vermicompost	5	9	0.00
extract	10	9	0.00
	15	8.4	6.67
Control	-	9	0.00
		CD	0.717
		SE (m)	0.242
		SE (d)	0.343
		CV	3.179

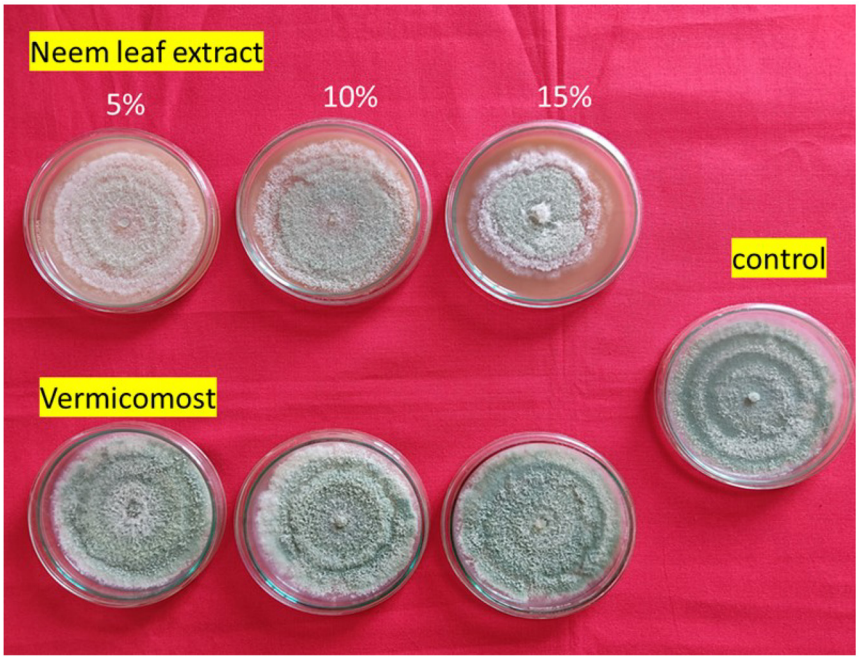

Plate 2. Compatibility of potential *Trichoderma* isolate with neem leaf extract and vermicompost at different concentrations after 4 days of incubation at 28 ± 2 °C.

all concentrations whereas, Vermicompost extract showed 6.67% inhibition at 15% and showed 100% compatibility with 5 and 10% concentrations (Table 2, Plate 2).

Therefore, Neem leaf extract at 15% and Vermicompost at 15% can be selected for IDM approach along with potential *Trichoderma.*

Bagwan (2010) observed that, neem oil (5%), neem leaves extract (10%), wild sorghum leaves extract (10%), neem cake, castor cake and mustard cake extract (10%) enhanced the growth of *Trichoderma.* Kumar *et al*. (2018) observed the compatibility of oil cakes with *Trichoderma viride* and the highest inhibition (19.2%) was recorded in neem cake followed by mustard cake (13.9%) and caster cake (7.6%) at 10% concentration.

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