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Preliminary Studies on Bio-efficacy of Different Botanical Extracts against *Sclerotium rolfsii* (Sacc.) Causing Collar Rot of Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Background: Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop. Collar rot caused by *Sclerotium rolfsii* Sacc. is an important devasting disease of chickpea in areas where seedling is exposed to high temperature and high moisture in the soil, affected seedlings turn yellow, which can be easily pulled out, which causes considerable damage to the plant stand. Botanical's control is very important aspects to minimize cost of cultivation and also to avoid environment pollution and health hazards. Thus, Botanicals extracts were tested for their efficacy against *S. rolfsii* for manage the disease.

Methods: Present investigation was undertaken for bio-efficacy of aqueous botanical extracts of different non-host plants *viz.,* neem seeds kernel extract (NSKE), neem leaves extract (NLE), tulsi leaves extract (TLE), datura leaves extract (DLE), aak leaves extract (ALE), lantana leaves extract (LLE), kaner leaves extract (KLE) and gajargass leaves extract (GLE) were evaluated *in vitro* at three concentrations *viz.,* 5, 10 and 15% against *S. rolfsii* causing collar rot of chickpea, by using poisoned food technique. Most effective *in vitro* evaluate botanical extracts further tested for their efficacy against management of the disease under pot experiment in net house.

Result: The result revealed that all the botanical extracts are significantly inhibited the growth of test organism at all the concentrations tested after 96 h of inoculation. The NSKE recorded significantly superior in inhibiting mycelial growth up to 47.22 and 59.44% at 10 and 15% concentration, respectively with a mean of 46.85%, followed by tulsi leaves extract with 43.61 and 56.11% at 10 and 15% concentration respectively with a mean of 43.61%. While, at 5% concentration both extracts at par statistically to each other. Lantana leaves extract is found statistically at par with tulsi leaf extract at all concentrations least inhibition was observed in kaner leaves extract under *in vitro* condition. Under pot experiments all the treatments proved significantly superior when compared with inoculated control. NSKE @ 15% concentration was found significantly superior to control the disease, when applied through seed treatment and integration of seed treatment and post emergence seedling drenching at 7 DAG.

Key words: Botanical extracts, Ccollar rot of chickpea, Drenching, Neem seeds kernel extract (NSKE), *Sclerotium rolfsii*.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is commonly known as "Bengal gram" or "Gram". Chickpea is a self-pollinated crop belonging to the sub-family: Papilionaceae, family: Leguminaceae. It is believed to be introduced into India from Western Asia. India ranks first in conditions of chickpea production and consumption in the world. In India, area under chickpea was 9.85 million hectares with a production of 11.99 million tonnes and productivity of 1217 kg/hectare. Whereas, in Rajasthan chickpea is an important winter legume crop, grown principally both in irrigated and rain-fed areas. It occupies an area 2.11 million hectares with a production of 2.32 million tonnes and productivity of 1099 kg/hectare during 2020-21 (Annonymus, 2021).

Despite the high total production and more nutritive value, productivity of chickpea was low due to many biotic and abiotic constraints. Among the biotic constraints Chickpea crop is attacked by 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and mycoplasma) from entire the world (Nene *et al.,* 1996). Among all, only a few of them have the potential to destroy crops. Collar rot caused by *Sclerotium rolfsii* Sacc. is an important disease in areas where seedling is exposed to high temperature and high moisture in the soil, affected seedlings turn yellow, which can be easily pulled out. *Sclerotium rolfsii* is an important soil borne and fast spreading fungal pathogen, which causes considerable damage to the plant stand. Seedling mortality in chickpea due to *S. rolfsii* has been reported to vary from 54.7 to 95.00% (Shrivastava *et al*., 1984). Under field conditions, *S. rolfsii* has been reported to cause 22 to 50 % reduction in yield of chickpea. Ghosh *et al*. (2013) surveyed four chickpea growing states of India *i.e*. Andhra Pradesh, Karnataka, Madhya Pradesh and Chhattisgarh and reported

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that losses from collar rot disease ranged from 7.1 to 10.5%. However, due to its soil borne nature and long survival of pathogen through sclerotia, it is difficult to manage through conventional method. The application of fungicides although effective, but is un-economical. They not only affect associated beneficial microbiome in soil but also are main source contributing towards environmental pollution. As such, use of alternative methods like eco-friendly and economical management through botanical extracts seems to be more appropriate to manage such soil borne diseases.

MATERIALS AND METHODS

Collection, isolation, pathogenicity and identification of *S. rolfsii*

Infected plants which showing typical collar rot symptoms were collected during month of October to December, 2018 from the chickpea fields of Agriculture Research Station, Ummedganj- (Kota) brings to laboratory for further studies. Isolation of fungus was carried through standard tissue isolation through infected plant parts and the pure culture of fungus was obtained by following hyphal tip culture under aseptic conditions was maintained on PDA slants at 4±1°C for further studies. Pathogenicity was proved through soil inoculation. Basis on culture characteristics fungus identified as *S. rolfsii*. Further, the identification of pathogen was confirmed from Indian Type of Culture Collection, Division of Plant Pathology, IARI, New Delhi (Ref. No. PP/3260; Date-25/03/2019).

Bio-efficacy of botanical extracts against the pathogen, *in vitro*

Anti-fungal activity of various non-host plant extracts *viz.,* neem seeds kernel extract (NSKE), neem leaves extract (NLE), tulsi leaves extract (TLE), datura leaves extract (DLE), aak leaves extract (ALE), lantana leaves extract (LLE), kaner leaves extract (KLE) and gajargass leaves extract (GLE) were evaluated *in vitro* at three concentrations *viz.,* 5, 10 and 15% against *S. rolfsii*, by poisoned food technique as suggested by Nene and Thapliyal, (2018).

Preparation of cold aqueous botanical extracts

Fresh sample of each above mention test plants were collected and washed first in tap water and then in distilled water. 100 g of fresh samples were crushed in a surface sterilized Pestle and mortar or mixer cum juicer by adding 100 ml sterile distilled water (1:1 w/v). The extract was filtered through double layer muslin cloth followed by Whatman's No. 1 filter paper and filtrate was considered as standard extract (100%) and used as stock solution.

Five, ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of sterilized molten PDA medium respectively, as to get 5, 10 and 15 % concentrations. The medium was thoroughly shaken for uniform mixing of the extract after adding the botanicals, to avoid the bacterial contamination a little amount of streptomycin antibiotic was added at the time of pouring media.

Near about 20 ml of medium was poured into each of 90 mm sterilized petri plates. Each plate was inoculated with 6 mm mycelial disc taken from the periphery of fresh fungal culture. The disc was placed upside down in the center of petri plate, so that the mycelium was in direct contact with the medium poisoned with requisite plant extracts at required concentration. Suitable control plates were maintained where in culture discs were inoculated into the center of potato dextrose agar plates without plant extracts.

Four replications were maintained for each treatment and incubated at 25±1°C till growth of colony touched the periphery in the control plate. Mean colony diameter in each case was recorded by taking the diameter of the colony in two directions. Radial growth of the fungus was measured and percent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947). The data were analysed statistically.

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

Where,
I = Per cent inhibition.
C = Growth in control.
T = Growth in treatment.

Pot experiment

Soil sterilization

For pot study the soil was sterilized by using formaldehyde by the following procedure. For this raised soil bed was prepared and watered the soil up to saturation level and left undisturbed for two days. After two days the soil was moistened by 4% formaldehyde solution (40 ml formaldehyde per liter of water) up to saturation level and covered by polythene sheet and kept undisturbed for five days. Polythene sheet was removed after five days and soil was exposed to open for seven days to remove the traces of formaldehyde present in soil. This soil was filled to the disinfected pots to carry out further studies.

Soil inoculation of pathogen

The sterilized soil was mixed with *Sclerotium rolfsii* which was isolated from diseased plants or mass cultured on Sand Sorghum Media. 10 g mass culture of *S. rolfsii* grown on sorghum seeds was added to upper 15 cm layer of soil in pots and mixed thoroughly. The mixed soil was placed in cemented pots. Apparently healthy seeds of chickpea (JG-14) were soaked overnight in water and surface sterilized with 0.1% $HgCl_2$ solution, washed thrice in tap water before sown in the pots with appropriate related treatment apply. Ten seeds were placed in one cemented pot after 24 hrs. of inoculation. Three replications were maintained for each treatment. These pots were kept in a net house. Moisture content in the soil was maintained to field capacity by adding

adequate amount of water regular interval. Proper isolation was maintained to avoid other pathogens. Without inoculated with test fungus was treated as control.

Efficacy of botanical extracts against disease under pot experiment

The botanical extracts tested *in vitro*; most effective botanical extracts were evaluated in pot experiment to find out the effective botanical extracts for collar rot management. To evaluate the fungicidal bio-efficacy of botanical extracts effective concentration of laboratory experiment was used for collar rot disease management in the pot experiment was carried out in net house through artificial soil inoculation method. For pot study stock aqueous solution prepared and required concentration was prepared by adding plant extract in 100 ml distilled water.

Treatments details

T_1 = Pre-emergence drenching (PED) 48 h after sowing by 15% concentration of neem* seeds kernel extract (NSKE) @ 50ml/pots.

T_2 = Pre-emergence drenching 48 h after sowing by 15% concentration of tulsi* leaves extract (TLE) @ 50 ml/pots.

T_3 = Pre-emergence drenching 48 h after sowing by 15% concentration of lantana leaves extract (LLE) @ 50 ml/pots.

T_4 = Seed treatment (ST) with 15% concentration of neem seeds kernel extract (NSKE) @ 0.4% /kg of seeds.

T_5 = Seed treatment with 15% concentration of tulsi leaves extract @ 0.4% /kg of seeds.

T_6 = Seed treatment with 15% concentration of lantana* leaves extract @ 0.4% /kg of seeds.

T_7 = T_4 + Post emergence seedlings drenching (PESD) by 15% concentration of neem seeds kernel extract (NSKE) @ 50ml/pots at 7 days after germination (DAG).

T_8 = T_5 + Post emergence seedlings drenching by 15% concentration of tulsi leaves extract @ 50 ml/pots at 7 days after germination (DAG).

T_9 = T_6 + Post emergence seedlings drenching by 15% concentration of lantana leaves extract @ 50ml/pots at 7 days after germination (DAG).

T_{10} = Inoculated with pathogen.

T_{11} = Only sterilized soil.

Statistical analysis of experimental data

Analysis and interpretation of the experimental data was done by using completely randomized design (CRD) for both as well as laboratory and pot experiments as suggested by Panse and Sukathme (1985).

Observation recorded

The percentage seed germination, pre-emergence seed rot and post-emergence seedling mortality were calculated by the formulae:

a.) Germination (%) = $\frac{\text{Number of seed germinated}}{\text{Total number of seed sown}} \times 100$

b.) Pre-emergence seed rotting % (PESR) = $\frac{\text{Number of seed germinated}}{\text{Total number of seed sown}} \times 100$

c.) Post-emergence seedling mortality % (PESM) = $\frac{\text{Number of seeding died}}{\text{Total number of seedling}} \times 100$

d.) Reduction (%) in PESR and PESM = $\frac{C-T}{C} \times 100$

Where,

C= Per cent seed rot/mortality in inoculated control pots and

T = Per cent rot/mortality in treated pots.

2. Yield per pot (g).

RESULTS AND DISCUSSION

***In-vitro* efficacy of botanical extracts on mycelial growth inhibition of the *S. rolfsii* on PDA by poisoned food technique**

The efficacy of eight botanical evaluated *in vitro* as described in “material and methods”. The data on percent growth inhibition of fungus is presented in Table 1 and Plate 1.

Clearly indicated that, the botanicals evaluated against the pathogen after 96 hrs. of inoculation, neem seeds kernel extract (NSKE) recorded significantly superior in higher mycelial growth inhibition of 47.22 and 59.44% at 10 and 15% concentration respectively with a mean of 46.85%, followed by tulsi leaves extract with 43.61 and 56.11% at 10 and 15% concentration respectively with a mean of 43.61%. While, 5% concentration is at par statistically to each other. Lantana leaves extract recorded 28.33, 42.08 and 53.33 per cent inhibition at 5, 10 and 15% concentrations with a mean of 41.25% is found statistically at par with tulsi leaf extract at all concentrations; datura leaves extract; aak leaves extract; gajargass leaves extract and neem leaves extract respectively showed moderate inhibition at 5, 10 and 15% concentrations. Least inhibition was observed in kaner leaves extract with 6.11, 16.67 and 22.78% inhibition at 5, 10 and 15% concentration respectively with a mean of 15.19 % compare to control. This differential anti fungitoxic activity of different extracts may be due to variation in composition of antifungal compounds in different plants. The effectiveness of neem seed kernel extract on mycelial growth inhibition of many fungi might be due to the presence of antifungal compounds like Azadirachtin. These results agree with Singh *et al.* (2007) observed that neem extract (*Azadirachta indica*) caused the maximum inhibition of mycelial growth and sclerotial production, its size and viability. Mahato *et al.,* (2018) advocated that *Allium sativum* was showed maximum inhibition of 35.31%, 68.50% and 84.89% of mycelia growth at 5, 10 and 20 percent concentration respectively followed by *Azadirachta indica* (31.67%, 65.61% and 80.86%). Sab *et al.,* (2014) reported that aqueous extract of agave at different concentrations, followed by henna leaves with 34.4, 71.3 and 90% at 5, 10 and 15 % concentration respectively and least mycelial

inhibition was observed in Tridax leaves extract (5.5%) and pongamia (7.1%). Farooq *et al.*, (2010) reported all plant species tested inhibited mycelial growth of the pathogen but maximum inhibition was recorded by *Azadirachta indica* (73.8%) followed by *Cassia fistula* (73.5%) and *Cannabis sativa* (67.1%). The minimum inhibition was showed by *Trigonella foenumgraecum* (34.3%) and *Cassia angustifolia* (36.3%). Butt *et al.,* (2016) reported that two important indigenous plants like *Alstonia scholaris* and *Azadirachta indica* leaf extract were more effective against *S. rolfsii* under *in-vitro* condition at different concentrations (1, 2, 3, 4 and 5%).

Efficacy of most effective *in-vitro* evaluate botanical extracts against disease under pot experiment

Results are presented in Table 2, Fig 1 and Plate 2 revealed that impact of treatment application method *viz.,* pre-emergence drenching, seed treatment and integration of both seed treatment and post emergence seedling drenching at 7 days after germination (7 DAG) of each most effective *in-vitro* evaluate botanical extracts as described earlier in "material and method" against collar rot disease of chickpea in pot experiment and effect on percent germination, percent pre-emergence seed rotting (PESR), percent reduction in PESR, per cent post emergence seedling mortality (PESM), pe rcent reduction in PESM, percent final plant population and yield per pot (g) were recorded. All the treatments proved significantly superior when compared with inoculated control. Maximum per cent reduction in PESR (63.64%) was recorded in NSKE was found significantly superior followed by lantana and tulsi leaves extracts with 54.55% reduction in PESR applied through seed treatment. Maximum percent reduction in PESM (37.50%) was recorded in NSKE found significantly superior followed by lantana leaves extracts (31.25%) reduction in PESM, applied through integration of seed treatment and post emergence seedling drenching at 7 DAG. Data recorded in Table 2, revealed that seed treatment alone reduced pre-emergence seed rotting while, integration of seed treatment and post

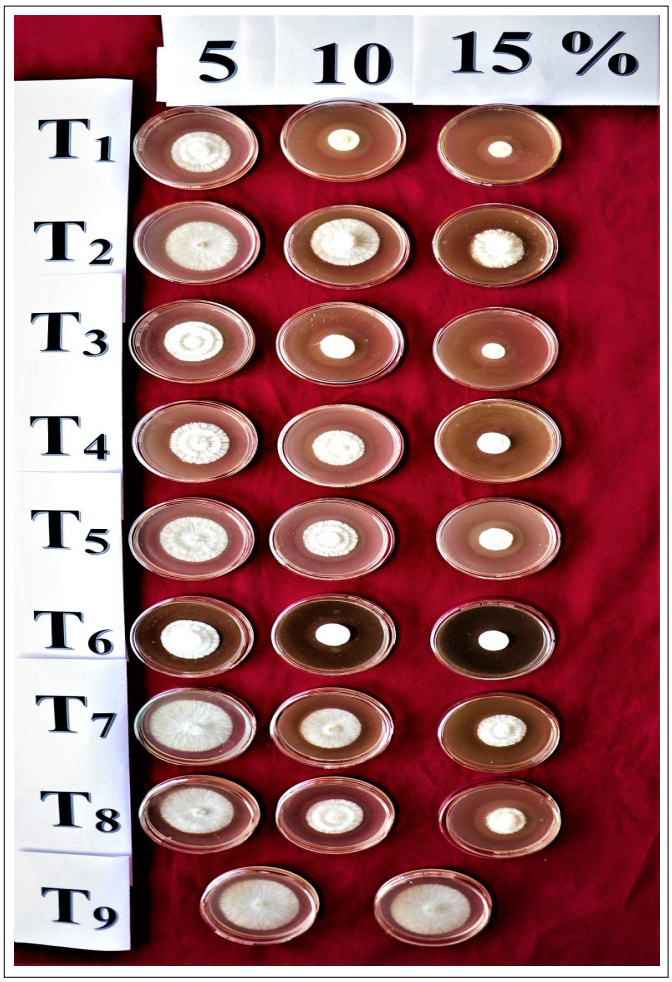

Plate 1: Efficacy of different botanical extracts on growth inhibition of *S. rolfsii*, 96 h after inoculation.

T_1: Neem Seeds Kernel Extract (NSKE), T_2: Neem leaves extract (NLE), T_3: Tulsi leaves extract (TLE), T_4: Datura leaves extract (DLE), T_5: Aak leaves extract (ALE), T_6: Lantana leaves extract (LLE), T_7: Kaner leaves extract (KLE), T_8: Gajargass leaves extract (GLE), T_9: Control.

emergence seedling drenching at 7 DAG also reduced PESM. Maximum per cent final plant population (58.33%) were observed in NSKE applied through integration of seed treatment and post emergence seedling drenching at 7 DAG followed by NSKE (50.00) applied through seed treatment. Among treated pots highest grain yield recorded (30.33 g/pot) in NSKE applied through integration of seed treatment and post emergence

Table 1: *In vitro* efficacy of botanical extracts on mycelial growth inhibition of *S. rolfsii,* by poisoned food technique at different concentrations (96 h after inoculation) at 25±1°C.

Treatment/ botanical extracts	Per cent mycelial growth inhibition of *S. rolfsii* at different concentrations*			
	5%	10%	15%	Mean
T_1: Neem seeds kernel extracts (NSKE)	33.89 (35.60)**	47.22 (43.41)	59.44 (50.45)	46.85 (43.15)
T_2: Neem leaves extract (NLE)	11.11 (19.35)	15.56 (23.22)	30.56 (33.55)	19.07 (25.37)
T_3: Tulsi leaves extract (TLE)	31.11 (33.89)	43.61 (41.33)	56.11 (48.51)	43.61 (41.24)
T_4: Datura leaves extract (DLE)	23.61 (29.07)	37.08 (37.51)	48.06 (43.89)	36.25 (36.82)
T_5: Aak leaves extract (ALE)	20.00 (26.28)	31.11 (33.89)	42.22 (40.52)	31.11 (33.56)
T_6: Lantana leaves extract (LLE)	28.33 (32.12)	42.08 (40.44)	53.33 (46.91)	41.25 (39.83)
T_7: Kaner leaves extract (KLE)	6.11 (13.96)	16.67 (23.82)	22.78 (28.25)	15.19 (22.01)
T_8: Gajargass leaves extract (GLE)	20.00 (26.52)	25.28 (30.16)	33.89 (35.58)	26.39 (30.75)
T_9: Control (Untreated)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Concentration mean	19.35 (24.09)	28.73 (30.42)	38.49 (36.41)	28.86 (30.30)
	Treatment	Concentration		T × C
S Em. ±	0.65	0.37		1.12
C.D. at 0.05%	1.82	1.05		3.14

*Average of four replications; **Figures in parentheses are Arc sine transformed values.

Plate 2: Best performed treatments along with application method under pot experiment.

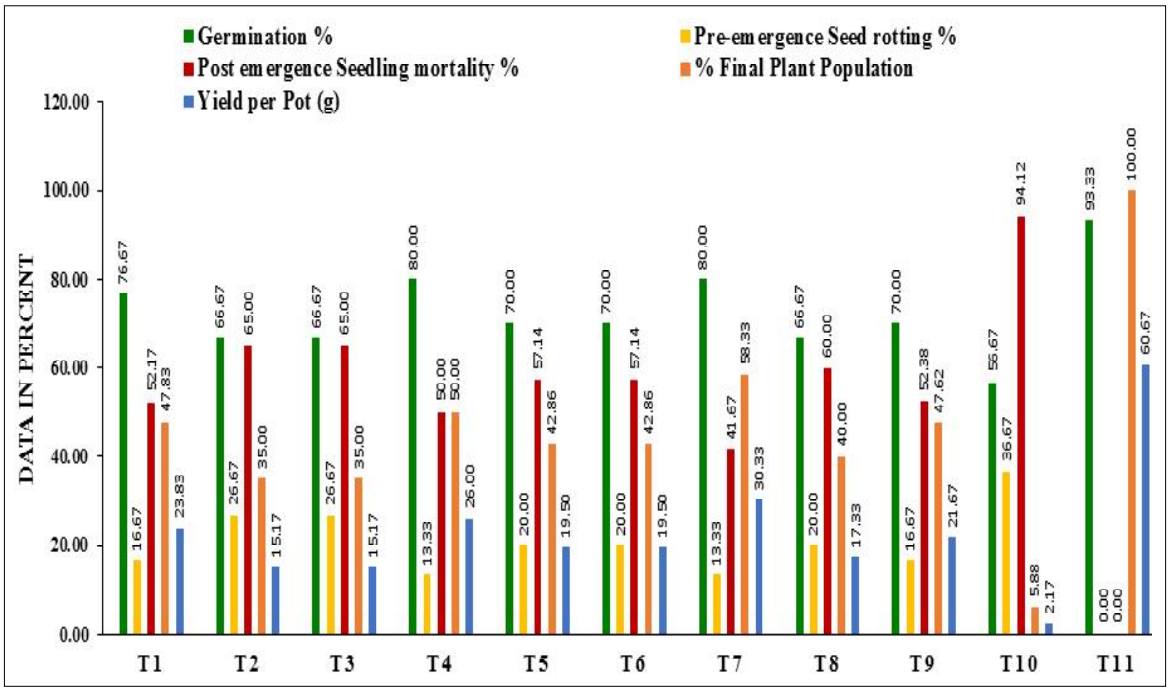

Fig 1: Efficacy of most effective *in-vitro* evaluate botanical extracts against disease under pot experiment.

Table 2: Efficacy of best *in-vitro* evaluate botanical extracts against collar rot of chickpea under pot experiments.

Treatment	No. of plant emerge	Germination (%)	No. of seed pre-emerge rotting	PESR %	% reduction in PESR	No. of seedling post emerge died	PESM %	% reduction in PESM	No. of final plant stand	% Final plant population	Yield per pot (g)
T_1	7.67*	76.67 (61.12)**	2.00	20.00 (26.57)	45.45 (42.39)	4.00	52.17 (46.25)	25.00 (30.00)	3.67	47.83 (43.75)	23.83
T_2	6.67	66.67 (54.74)	2.67	26.67 (31.09)	27.27 (31.48)	4.33	65.00 (53.73)	18.75 (25.66)	2.33	35.00 (36.27)	15.17
T_3	6.67	66.67 (54.74)	2.67	26.67 (31.09)	27.27 (31.48)	4.33	65.00 (53.73)	18.75 (25.66)	2.33	35.00 (36.27)	15.17
T_4	8.00	80.00 (63.43)	1.33	13.33 (21.42)	63.64 (52.91)	4.00	50.00 (45.00)	25.00 (30.00)	4.00	50.00 (45.00)	26.00
T_5	7.00	70.00 (56.79)	1.67	16.67 (24.09)	54.55 (47.61)	4.00	57.14 (49.11)	25.00 (30.00)	3.00	42.86 (40.89)	19.50
T_6	7.00	70.00 (56.79)	1.67	16.67 (24.09)	54.55 (47.61)	4.00	57.14 (49.11)	25.00	3.00 (30.00)	42.86	19.50 (40.89)
T_7	8.00	80.00 (63.43)	1.33	13.33 (21.42)	63.64 (52.91)	3.33	41.67 (40.20)	37.50 (37.76)	4.67	58.33 (49.80)	30.33
T_8	6.67	66.67 (54.74)	2.00	20.00 (26.57)	45.45 (42.39)	4.00	60.00 (50.77)	25.00 (30.00)	2.67	40.00 (39.23)	17.33
T_9	7.00	70.00 (56.79)	1.67	16.67 (24.09)	54.55 (47.61)	3.67	52.38 (46.36)	31.25 (33.99)	3.33	47.62 (43.64)	21.67
T_{10}	5.67	56.67 (48.83)	3.67	36.67 (37.27)	0.00 (0.00)	5.33	94.12 (75.96)	0.00 (0.00)	0.33	5.88 (14.04)	2.17
T_{11}	9.33	93.33 (75.04)	-	-	-	-	-	-	9.33	100.00 (90.00)	60.67
		Germination	**Pre-emergence seed rotting**			**Post emergence Seedling mortality**		**Final plant stand**			**Yield per pot**
S Em. ±		0.28	0.62			0.32		0.72			1.85
C.D. at 0.05%		0.82	1.81			0.95		2.10			5.42

*Average of three replications; **Figures in parentheses are Arc sine transformed values.

seedling drenching at 7 DAG followed by NSKE (26.00 g/pot) applied through seed treatment. While in inoculated control grain yield recorded were (2.17 g/Pot). All the treatments were found to control collar rot disease significantly over inoculated control. Botanical's control is very important aspects to minimize cost of cultivation and also to avoid environment pollution and health hazards. Botanicals were tested for their efficacy against *S. rolfsii* by many workers. This in turn may indicate about the use of such botanicals in plant disease control. The results of the present studies are in confirmatory to Okereke and Wokocha (2006) who reported that, the inhibition of damping-off disease of tomato incited by *S. rolfsii* was highest with soil drenching with neem seed extract (62.4%) followed by ginger (57.4%). Gupta *et al.*, (2012) reported the efficacy of percentage inhibition at 42 h on radial growth at 10% was 100% in garlic, followed by neem (97.0%). The maximum disease control was observed in garlic (76.7%), followed by neem (75.7%) against *S. rolfsii* [*Athelia rolfsii*], causing collar rot of chickpea. Mahato *et al.,* (2018) reported *Azadirachta indica* effective against collar rot disease at 20 per cent concentration by reducing 64.69% and 67.26% disease incidence respectively followed by *Allium sativum* (60.34% and 62.65%) and least reduction of disease incidence (29.38% and 25.30%) respectively recorded in *Ocimum sanctum* in two years of *in vivo* experiments.

CONCLUSION

Among the botanicals evaluated, NSKE was significantly superior in controlling collar rot of chickpea. When applied through seed treatment and integration of seed treatment and post emergence seedling drenching at 7 DAG. The results however need field evaluation before these recommended to farmers.

Conflict of interest: None.

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