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MANAGEMENT OF COLLAR ROT OF CHICKPEA USING DIFFERENT SOLVENT-BASED PLANT EXTRACTS

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ABSTRACT

Chickpea is an important legume crop in India and contributes a significant portion to pulse production. Chickpea is attacked by various soil borne plant pathogens in which collar rot is a major problem in the pulse growing region, causing considerable loss to the growers. An experiment was conducted at BTC, College of Agriculture and Research Station, Sarkanda, Bilaspur (IGKV), Chhattisgarh to evaluate the efficacy of different solvent-based plant extracts against *Sclerotium rolfsii in vitro* as well as *in vivo* conditions. Total four plant parts extracts using four different solvents viz. acetone, ethanol, methanol and petroleum ether were evaluated against *Sclerotium rolfsii*. Maximum suppression of fungus was recorded in Ethanol-based neem extract (93.89%) followed by Methanol based neem extract (93.15%) and cow urine-based neem extract (66.48%) under *in vitro* conditions. The efficacy of plant extracts was also tested under *in vivo* condition and Acetone based neem extracts (76.67%) showed a significantly higher germination percentage followed by Cow urine-based neem extracts (74.67%), Cow urine-based plant extracts mixture (74.00%) and Ethanol based plant extracts mixture (60.00%) in sick soil. Cow urine + Hexaconazole 5% SC @0.05% (8.67%) was most effective in reducing pre-emergence seedling mortality followed by Acetone based neem extracts (23.33%), Cow urine-based neem extracts (25.00%), Cow urine-based plant extracts mixture (26.00%) and Ethanol based plant extracts mixture (39.33%). Similarly, Cow urine-based plant extracts mixture + Hexaconazole 5% SC @0.05% (5.03%) and Hexaconazole 5% SC @0.05% (7.73%) were significantly effective (4.80% - 35.23%) in suppressing the post-emergence seedling mortality (PESM) due to collar rot of chickpea over control.

Key words : Chickpea, Plant extract, Neem, *Sclerotium rolfsii*, Collar rot.

Introduction

Chickpea (*Cicer arietinum* L.), an important pulse, is a good source of carbohydrates and proteins. Sclerotium rolfsii is a soil-born pathogen that causes collar rot disease in chickpea resulting in significant yield losses. Globally, chickpea is grown in an area of 148.42 lakh hectares with a production of 150.84 lakh tonnes and productivity of 1016 kg per hectare (FAO, 2020). In India, chickpea takes first position in total pulse production. During 2020-2021, Chhattisgarh contributed 3.06% of total chickpea production with a productivity of 2.23 kg per hectare (Anonymous, 2021).Collar rot of chickpea caused by *Sclerotium rolfsii* (teleomorph: *Athelia rolfsii*) is a devastating soil-borne disease causing heavy economic losses to the chickpea crop (Kokub *et al*., 2007). The disease primarily affects the early growth stages of the plant often before pod formation causing younger seedlings to turn yellow and older seedlings to dry without collapsing. If affected seedlings are uprooted from moist soil in the earlier stages of infection rape seed like sclerotia can be seen on roots (Nene *et al*., 2012). Under field conditions *S. rolfsii* has been reported to cause 22 to 50 per cent reduction in yield of chickpea (Ghosh *et al*., 2013). Wavare *et al*. (2017) evaluated methanol, acetone, dichloromethane and aqueous extracts of four flowers marigold (*Tagetes erecta*), *Gaillardia* sp.

Chrysanthemum sp. and *Calotropis* sp. *in vitro* and *in vivo* against *Sclerotium rolfsii*. They found reduced biomass production of *S. rolfsii* by 85, 87 and 78% in Parthenium, Jatropha and Annona bio wash. In pot trials, the collar rot of chickpea was managed through the use of a fungicide namely, mancozeb and Neem dry leaf biomass. Disease highly occurred in positive control, whereas negative control was disease free. The disease incidence was reduced significantly in Neem dry leaf biomass and mancozeb-treated soils. The fungicide gave better results than the dry leaf biomass. There are reports that fungicides are highly effective for the control of target fungus *S. rolfsii* (Shirsole *et al.*, 2019)

Materials and Methods

Preparation of plant extracts

Collection of cow urine and preparation of cow urine-based plant extracts

The cow urine was collected in the morning from an indigenous breed of cow in a sterile container, brought to the laboratory immediately and urine were filtered aseptically. Cow urine-based extraction of selected plants was prepared using technique given by Rakesh *et al.* (2013).

Collection of plants leaves

Fresh leaf was collected randomly from the medicinal plant nursery maintained at Agriculture College Campus, Bilaspur (Chhattisgarh). The following plant parts used for under the present investigation.

Organic solvent extracts (acetone, ethanol, methanol and petroleum ether) were prepared by incorporating the chopped plant materials in equal quantity of respective solvents (1:1 W/V). Plant parts were mixed well in solvents using pestle mortar and kept open for overnight to evaporate the organic solvents. Remaining content were filtered and the filtrates were subjected to low-speed centrifugation at 5000 rpm for five min using a centrifuge (Priya and Ganjewala, 2007). The supernatant thus obtained were collected gently with the help of a micropipette and transferred in glass vials. Vials were kept open overnight at room temperature for the evaporation of any residual solvent. Crude plant extracts thus obtained were regarded as standard stock solution (100%) and stored at 4ºC for future use (Tiwari *et al.*, 2005).

Plant leaf extracts of medicinal plants i.e.- *Azadirachta indica* (Neem), *Millettia pinnata* (karanj), *Cascabela thevetia* (kanher), *Calotropis gigantea* (Ankh), were prepared in different solvent and used under *in vitro* and *in vivo* experiments.

Evaluation of Plant extracts against *S. rolfsii* using poison food techniques

To evaluate the *in vitro* antifungal efficacy of crude plant extracts 'poisoned food technique' was applied (Grover and Moore, 1962; Mishra and Tiwari, 1992; Nene and Thapliyal, 2000). 100 ml of Potato Dextrose Agar medium (PDA) was prepared in 250 ml Erlenmeyer flask and sterilized in autoclave. Crude plant extracts were used at two concentrations (5% and 10%) for this experiment. Two different concentrations of extracts were prepared by mixing aseptically 5 ml and 10 ml of stock solution (100%) in 100 ml of semisolid PDA medium at a temperature of 40°-45°C (Tiwari *et al.*, 2005). Media with same volume of sterile distilled water and different extraction solvents served as control and solvent control respectively. Hexaconazole at 100 ppm concentrations were used to compare the efficacy of plant extracts and regarded as fungicide control for *S. rolfsii* respectively. In general, antibiotic Streptomycin (100 ppm) was added to semisolid PDA medium before pouring Petri plates to check any bacterial contamination.

Petri plates with gelled medium were aseptically inoculated at the center with mycelial dies of 7 mm diameter taken from 96 hrs. old culture of *S. rolfsii* Plates were incubated at 26 +1°C in BOD incubator and mycelial growth were recorded. Colony diameters were recorded twice perpendicularly. Observations for *S. rolfsii* were taken 96 hrs after inoculation using a transparent millimeter ruler. Percentage inhibition of mycelial growth (Verma and Kharwar, 2006) was calculated by the following formula.

The per cent inhibition of mycelial growth of the pathogen were measured by the formula (Vincent, 1927),

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

Table 1 : List of plants used against *Sclerotium rolfsii*.

S. no.	Plant name	Botanical name	Family	Part used
1	Neem	*Azadirachta indica* (L.)	Meliaceae	Leaf
2	Karanj	*Millettia pinnata* (L.)	Fabaceae	Leaf
3	Kanher	*Cascabelathevetia* (L.)	Apocynaceae	Leaf
4	Calotropis	*Calotropis gigantea* (L.)	Apocynaceae	Leaf

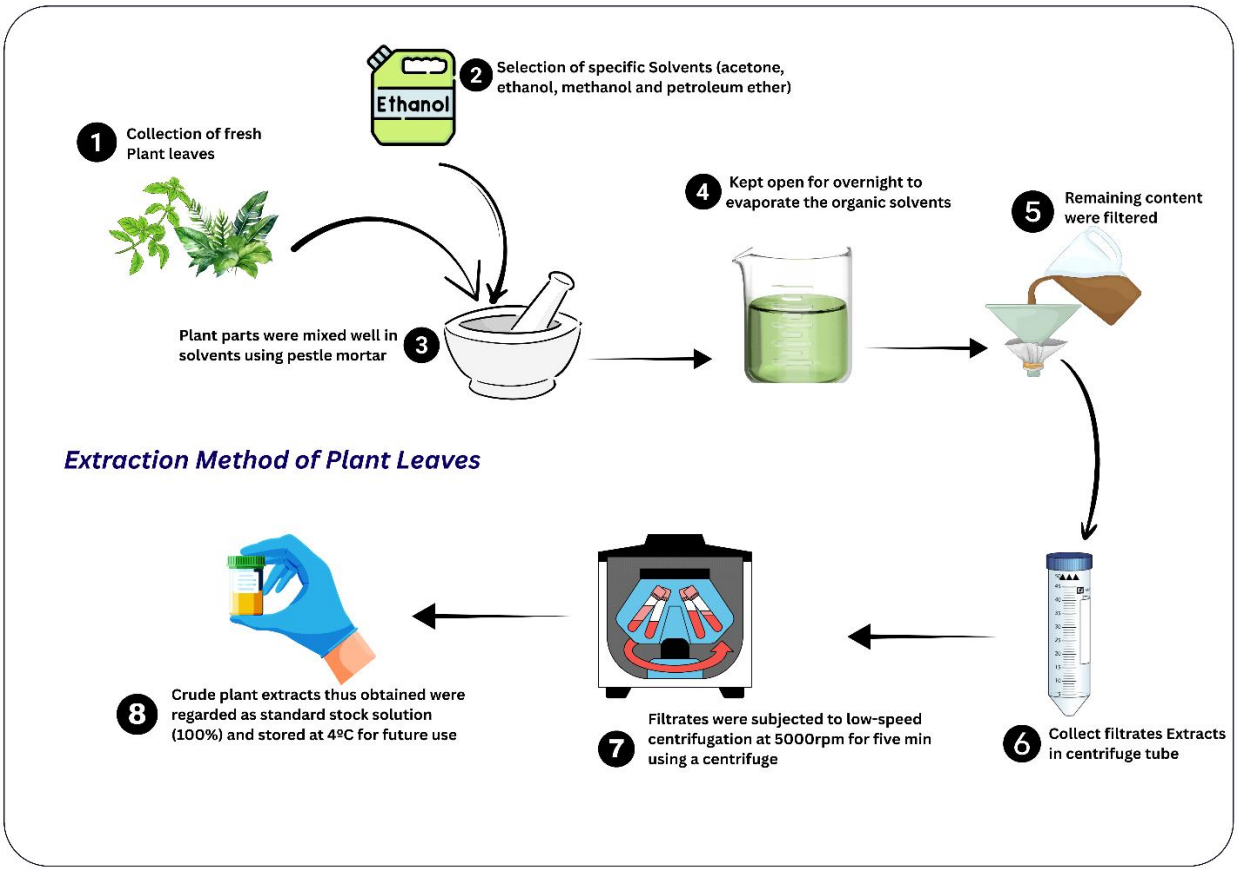

Fig. 1 : Extraction method of plant leaves. (1) Collection of fresh Plant leaves. (2) Selection of specific Solvents (acetone, ethanol, methanol and petroleum ether). (3) Plant parts were mixed well in solvents using pestle mortar. (4) Kept open for overnight to evaporate the organic solvents. (5) Remaining content were filtered. (6) Collect filtrates Extracts in centrifuge tube. (7) Filtrates were subjected to low-speed centrifugation at 5000 rpm for five min using a centrifuge. (8) Crude plant extracts thus obtained were regarded as standard stock solution (100%) and stored at 4ºC for future use.

Where, I = Per cent inhibition of mycelial growth,

C= radial growth of fungus in control plate (mm)

T=radial growth of fungus on treatment plate

Development of formulations of different solvent-based plant extracts and testing their efficacy as seed treatment for the management of collar rot of chickpea

Inoculation of pots

Pots (28 cm diameter) containing sterilized sandy loam soil, were further inoculated with two weeks old culture of *Sclerotium rolfsii* (multiplied on sorghum grain medium) @ 25g/kg soil and allowed to incubate for fifteen days. Pots were regularly observed for the Sclerotia formation and top layer of soils along with Sclerotia were removed from the pot after Sclerotia formation and mixed with the soil a definite proportion. Population of Sclerotia were determined by the adding 10 gm of sick soil into water. Fresh pot further inoculated with 100g of sick soil and covered and mixed with the soil with the definite proportion @ (580) number of sclerotia. Treated chickpea seeds were sown (variety RVG- 202) in pots (28 ×28 cm) @ 50 seeds / pot.

Observation recorded

- Observations on seed germination and pre-emergence seedling mortality were recorded seven days after sowing while post emergence seedling mortality (PESM) was recorded 30 days after sowing.
- The percentage seed germination, pre-emergence seedling mortality and post emergence seedling mortality were calculated using following formula.

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

Pre emergence seed mortality (%)

$$= \frac{\text{Number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

Post emergence seed mortality (%)

$$= \frac{\text{Number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

$$\text{Reduction } (\%) \text{ in PESM} = \frac{C - T}{C} \times 100$$

Where, T = Per cent mortality in treatment pots and

C = Per cent mortality in untreated control pots.

Results and Discussion

Evaluation of plant extracts against *S. rolfsii* using poison food techniques

Data on per cent inhibition of mycelial growth of *Sclerotium rolfsii* indicated that the significantly higher per cent of inhibition (14.26% -100.0%) was recorded from all the treatments used at 10% concentration. complete 100% per cent inhibition of mycelial growth *Sclerotium rolfsii* was recorded from Hexaconazole 5% SC (0.1%), Methanol based plant extract mixture (10%) and Ethanol based plant extract mixture (10 %) at 10 % concentration. Other treatments i.e. Ethanol-based neem extract (93.89%), Methanol based neem extract (93.15%) and hexaconazole 5 % SC @0.05% (90.74 %) were highly effective followed by cow urine-based neem extract (66.48%), cow urine-based plant extract mixture (65.56%), pure cow urine (59.81%), hot water-based plant extract mixture (57.04%), hot water-based neem extract (55.93%), Acetone based plant extract mixture (45.19%) and Acetone based neem extract (40.19%) in inhibiting per cent mycelial growth of *S rolfsii* at 10 per cent concentration.

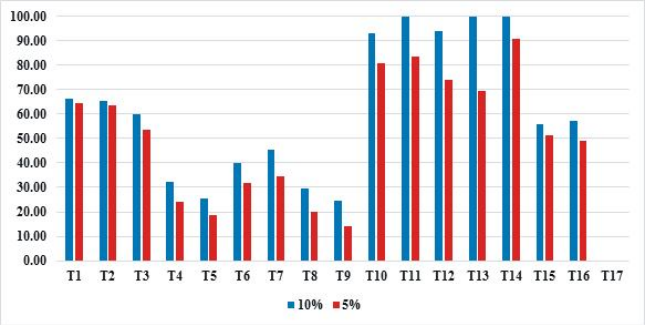

Fig. 2 : *In vitro* efficacy of different solvent-based plant extracts on per cent inhibition of *Sclerotium rolfsii*.

Similarly, *i.e.* Methanol based neem extract (80.74%), Ethanol based neem extract (73.89%), cow urine-based neem extract (64.44%), cow urine-based plant extract mixture (63.33%), Cow urine-based plant extract mixture (53.52%), Cow urine-based neem extract (51.11%), Acetone based plant extract mixture (34.44%) and Acetone based neem extract (31.67%) were found significantly effective in inhibiting per cent mycelial growth of *S. rolfsii*.

Development of formulations of different solvent-based plant extracts and testing their efficacy as seed treatment for the managent of collar rot of chickpea

The data on different solvent-based plant extracts used as seed treatments against collar rot incidence in chickpea are presented in Table 3. Results indicated that all the treatments were significantly superior (60.67% – 98.00%) in increasing germination percentage and suppressing pre and post emergence mortality due to collar rot of chickpea over control. Seeds treated with

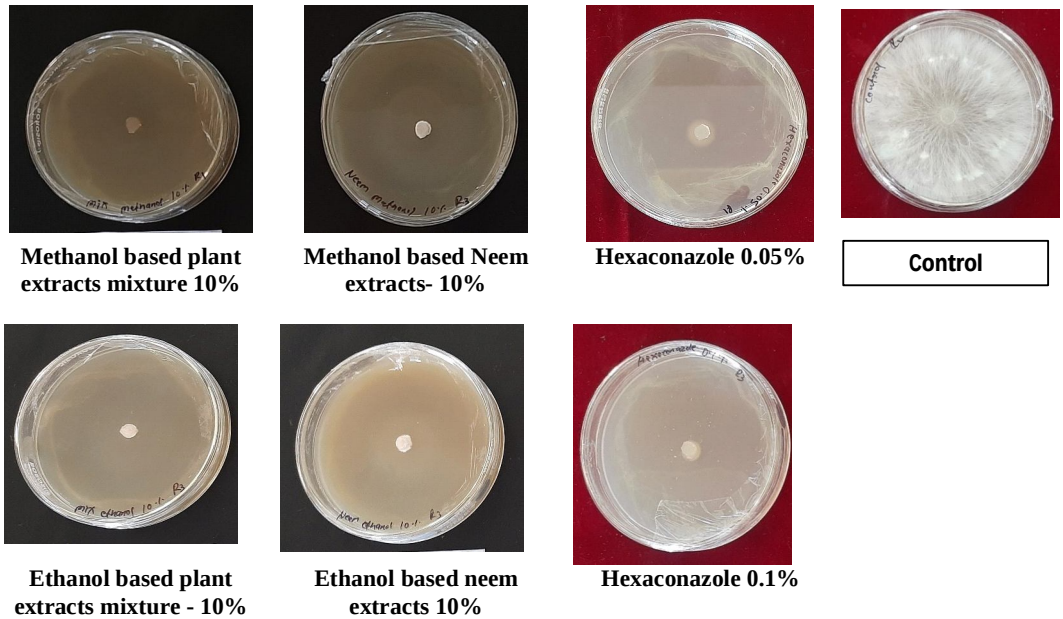

Plate 1 : *In vitro* efficacy of different solvent-based plant extracts against mycelial growth *Sclerotium rolfsii*.

Table 2 : *In vitro* efficacy of different solvent-based plant extracts against mycelial growth *Sclerotium rolfsii.*

	Treatments	Mycelial growth (mm)			Per cent inhibition		
		5 %	10 %	Mean	5 %	10 %	Mean
T_1	Cow urine-based neem extract	33.00(38.66)	31.17(37.39)	38.02	64.44(49.63)	66.48(50.99)	50.31
T_2	Cow urine-based plant extract mixture	34.00(39.33)	32.00(37.97)	38.65	63.33(48.91)	65.56(50.37)	49.64
T_3	Pure cow urine	41.83(40.30)	36.17(36.96)	38.63	53.52(47.02)	59.81(50.67)	48.84
T_4	Hot water-based calotropis extract	68.50(55.86)	61.17(51.46)	53.66	23.89(29.26)	32.04(34.45)	31.85
T_5	Hot water-based karanj extract	73.33(58.98)	67.17(55.05)	57.01	18.52(25.31)	25.37(30.23)	27.77
T_6	Acetone based neem extract	61.50(51.66)	53.83(47.21)	49.43	31.67(34.23)	40.19(39.32)	36.77
T_7	Acetone based plant extract mixture	59.00(50.20)	49.33(44.62)	47.41	34.44(35.91)	45.19(42.23)	39.07
T_8	Petroleum ether-based neem extract	71.83(57.97)	63.33(52.76)	55.36	20.19(26.65)	29.63(32.93)	29.79
T_9	Petroleum ether-based plant extract mixture	77.17(61.46)	67.83(55.47)	58.46	14.26(22.18)	24.63(29.71)	25.95
T_{10}	Methanol based neem extract	17.33(24.60)	6.17(14.37)	19.49	80.74(63.97)	93.15(74.84)	69.41
T_{11}	Methanol based plant extract mixture	15.00(22.77)	0.00(0.23)	11.50	83.33(65.92)	100.0(89.77)	77.84
T_{12}	Ethanol based neem extract	31.50(30.11)	5.50(13.56)	26.83	73.89(57.23)	93.89(75.69)	69.46
T_{13}	Ethanol based plant extract mixture	27.50(31.62)	0.00(0.23)	15.93	69.44(56.45)	100.0(89.77)	73.11
T_{14}	Hexaconazole 5% SC	8.33(16.77)	0.00(0.23)	8.50	90.74(72.30)	100.0(89.77)	81.03
T_{15}	Hot water-based neem extract	44.00(41.55)	39.67(39.04)	40.29	51.11(45.64)	55.93(48.40)	47.02
T_{16}	Hot water-based plant extract mixture	45.83(42.61)	38.67(38.44)	40.52	49.07(44.47)	57.04(49.05)	46.76
T_{17}	Control	90.00(71.57)	90.00(71.57)	71.57	0.00(0.23)	0.00(0.23)	0.23
	Mean	**42.70**	**33.91**		**43.25**	**52.85**	
		Factor A	**Factor B**	**A×B**	**Factor A**	**Factor B**	**A×B**
	C.D. 5%	**0.56**	**1.64**	**2.32**	**0.66**	**1.92**	**2.71**
	SE(m) ±	**0.47**	**0.02**	**0.47**	**0.55**	**0.03**	**0.55**
	C.V. %	**3.71**	**3.46**				

* Figures in the parentheses indicate arc sine transformed

* (Plant extracts mixture- neem leaf + karanj leaf + calotropis leaf + kanher leaf)

Table 3 : Effect of seed treatment with different solvent-based plant extracts on seed germination, pre-emergence mortality and post emergence mortality due to collar rot of chickpea.

	Treatments	Per cent germination	Pre-emergence seedling mortality %	Per cent reduction in PESM	Post emergence seedling mortality %	Per cent reduction in PESM
T_1	Pure cow urine	62.67(52.33)	37.33(37.64)	41.12	29.87(33.11)	60.58
T_2	Cow urine-based neem extracts	74.67(59.83)	25.00(29.98)	61.67	23.30(28.84)	69.25
T_3	Cow urine-based plant extracts mixture	74.00(59.36)	26.00(30.63)	60.00	19.00(25.82)	74.92
T_4	Acetone based neem extracts	76.67(61.51)	23.33(28.86)	64.45	21.33(27.47)	71.85
T_5	Acetone based plant extracts mixture	73.33(59.05)	26.67(31.05)	58.88	24.53(29.67)	67.63
T_6	Methanol based neem extracts	62.00(52.01)	38.00(38.02)	40.00	29.60(32.91)	60.93
T_7	Methanol based plant extracts mixture	68.67(56.00)	31.33(34.02)	51.12	31.57(34.11)	58.33
T_8	Ethanol based neem extracts	70.00(56.81)	30.67(33.59)	52.22	18.90(25.70)	75.06
T_9	Ethanol based plant extracts mixture	60.67(51.14)	39.33(38.82)	37.78	35.23(36.38)	53.50
T_{10}	Hexaconazole 5% SC (0.1%)	98.00(83.42)	2.00(7.94)	98.90	4.80(12.45)	96.70
T_{11}	Hexaconazole 5% SC (0.05%)	96.00(80.65)	4.00(11.47)	96.67	7.73(15.99)	89.80
T_{12}	Cow urine-based plant extracts mixture + Hexaconazole 5% SC (0.05%)	97.33(82.54)	2.67(8.92)	98.88	5.03(13.30)	95.32
T_{13}	Pure cow urine + Hexaconazole 5% SC (0.05%)	91.33(73.79	8.67(17.10)	88.88	8.53(16.94)	88.74
T_{14}	Control	38.00(38.03)	62.00(51.92)	0.00	75.77(60.57)	0.00
	SE m ±	**2.97**	**0.99**	**-**	**1.30**	**-**
	CD 5%	**8.65**	**2.91**	**-**	**3.79**	**-**
	CV	**8.32**	**6.05**	**-**	**8.13**	**-**

*Per cent values in parentheses are arcsine transformed

* (Plant extracts mixture- neem leaf + karanj leaf + calotropis leaf + kanher leaf).

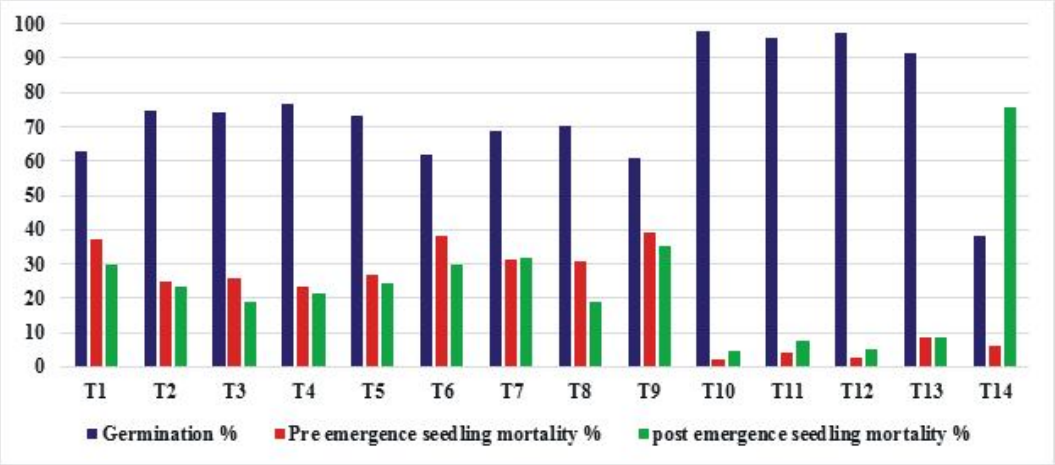

Fig. 3 : Effect of seed treatment with different solvent-based plant extracts on seed germination and post emergence mortality due to collar rot of chickpea.

Hexaconazole 5% SC (0.1%) (98%) followed by combination of cow urine-based plant extracts mixture + Hexaconazole 5% SC (0.05%) (97.33%), pure cow urine + Hexaconazole (0.05%) (91.33%) and Hexaconazole 5% SC (0.05%) (96.00%) were found significantly more effective in over all other treatments including control (38.00) in increasing percent germinations and found statistically at par with each other. Seed treated with other solvent based plant extracts i.e. Acetone based neem extracts (76.67%), Cow urine-based neem extracts (74.67%), Cow urine-based plant extracts mixture (74.00%) and Ethanol based plant extracts mixture (60.00%) had have significantly higher percents germination compared to control (38.00%).

Data presented in Table 3 indicated that all the treatments were significantly effective (2.00% - 39.33%) in suppressing the pre-emergence seedling mortality (PESM) due to collar rot of chickpea over control. Results indicated that significantly lowest pre-emergence seedling mortality (PESM) was observed in treatment Hexaconazole 5% SC @ 0.1% (2.00%) followed by Cow urine-based plant extracts mixture + hexaconazole @ 0.05% (2.67%) and Hexaconazole 5% SC @ 0.05% (4.00%) over other treatments including control and found more effective statistically at par with each other. Other solvent based plant extracts *i.e.* pure cow urine + Hexaconazole 5% SC @ 0.05% (8.67%), Acetone based neem extracts (23.33%), Cow urine-based neem extracts (25.00%) and Cow urine-based plant extracts mixture (26.00%) followed by Ethanol based plant extracts mixture (39.33%) and Methanol based neem extracts (38.00%) compared to control (62.00%).

Results indicated that the highest reduction per cent in pre-emergence seedling mortality (PESM) was observed in the treatment Hexaconazole 5% SC used @ 0.1% (98.90%) followed by Cow urine-based plant extracts mixture + hexaconazole @0.05% (98.88%), Hexaconazole 5% SC @0.05% (96.67%) and Pure cow urine + Hexaconazole 5% SC @0.05% (88.88%) compared to control (0.0%).

Similarly, all the treatments were significantly effective (4.80% - 35.23%) in suppressing the post-emergence seedling mortality (PESM) due to collar rot of chickpea over control. The lowest post emergence seedlings mortality percentage was observed in Hexaconazole @0.1% (4.80%) followed by Cow urine-based plant extracts mixture + Hexaconazole 5% SC @0.05% (5.03%) and Hexaconazole 5% SC @0.05% (7.73%) which were also found significantly more effective over other treatments including control and statistically at par with each other. Other solvent based plant extracts i.e. pure cow urine + Hexaconazole 5% SC @0.05% (8.53%), Ethanol based neem extracts (18.90%), Cow urine-based plant extracts mixture (19.00%) and Acetone based neem extracts (21.33%) also found significantly effective for suppressing the post-emergence seed mortality over control (75.77%).

Highest reduction per cent in post-emergence seedling mortality (PESM) was observed in the treatment Hexaconazole 5% SC @0.1% (96.70%) followed by Cow urine-based plant extracts mixture + hexaconazole @0.05% (95.32%), Hexaconazole 5% SC @0.05% (89.80%) and Pure cow urine + Hexaconazole 5% SC @0.05% (88.74%) compared to control (0.0%).

Suggestions

There is need of isolation and quantification of active chemical psent in different solvent-based plant extracts. More number of different solvent-based plant extract

need to study against different plant pathogens. Large number of field trails may be conducted to evaluate the efficacy of combination of fungicide and cow urine-based plant extracts for the control of soil borne and foliar diseases i.e. collar rot of chickpea, sheath blight of rice, powdery mildew of okra and *Cercospora* leaf spot of okra etc.

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