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In vitro evaluation of bio-efficacy of different concentrations of fungicides against *S. rolfsii*

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Abstract
Collar rot of chickpea is major disease of chickpea which causes severe loss to chickpea crop under field condition. In present study evaluation of different fungicides at different concentrations against *Sclerotium rolfsii*. Among seven fungicides tested the maximum 100% inhibition was observed in treatment T_1 (Difenconazole) (15, 20, 25) µl and T_2 (Hexaconazole) (100, s150, 200) µl respectively.

Keywords: Fungicides, *S. rolfsii*, bio-efficacy

Introduction
Chickpea (*Cicer arietinum* L.), is a well-known major leguminous crop all over the world (Knights *et al*., 2007) [8]. First cultivation of this crop has been started in areas of south eastern region but now it is cultivated in semi-arid regions as well (Agarwal *et al*., 2012) [1]. Being the major source of protein in our diet it also plays major role in the improvement of soil fertility thorough nitrogen fixation activity (Hossain *et al*., 2010) [7]. Now a days growing demand of chickpea is increasing due to its nutritive value as it is a rich source of carbohydrate and free of cholesterol (Chibbar *et al*., 2010) [5].
Sclerotium rolfsii Sacc, the causal agent of collar rot disease in chickpea is now considered as serious threat to chickpea ecosystem that may cause around 60-90% mortality of the crop at seedlings stage under favourable environmental conditions (Al-Askar *et al*., 2013) [2]. Due to formation of sclerotia and its persistent as resting structure in the soil by pathogen its management is very challenging (Sennoi *et al*., 2013) [10]. Wide host range of this pathogen has led to attack of various hosts and cause many diseases like stem rot, seedling blight, collar rot, damping off, sclerotium wilt and charcoal rot (Gopalkrishnan *et al*., 2005) [6].
Myecilial growth of the fungi has been reported to be restricted by different fungicides (Zamora *et al*., 2008) [13]. Thiram and captan can successfully inhibit *in vitro* growth of *S. rolfsii*. Many combination product of fungicides *viz*., thiram and quintozene has been proved to be best in inhibiting the sclerotial formation of fungi (Yaqub and Shahzad, 2006) [12]. Considering the above point's present work was done to assess potential of different fungicides groups against growth of *S. rolfsii* under *in vitro* condition.

Material and Methods
To test the *in vitro* efficacy of seven fungicides poisoned food technique was employed with three different concentrations of fungicides against *S. rolfsii.* Test fungicides was diluted in required quantity with autoclaved PDA (Potato Dextrose Agar) medium in conical flasks. In order to facilitate uniform mixture flask containing fungicidal medium was shaken well and 20 ml well mixed PDA with each treatment was poured in sterile Petri plate. With the help of sterile cork borer, the inoculums disc of 5 mm diameter was cut from 7 days old pure culture and placed at the centre on Petri plate containing solidified fungicidal medium. Three replications of each treatment were kept for observation. Poured plate without fungicide served as control. Incubation of inoculated plates were incubated at (28±2°C) temperature. Observation was done with three repetitions against the pathogen. The inhibition per cent over control was calculated by using formula of Vincent, 1947.

Results and Discussion

The efficacy of different six fungicides and one antibiotics (Difenconazole 25% EC, Hexaconazole 5% EC, Tebuconzole 25.9% EC, Kreoxime Methyl 44.3% EC, Propiconazole 25% EC, Thifluzamide 24% SC and Validamycin 3% L) were evaluated *in vitro* at three different concentration of each fungicide i.e.15 µl, 20 µl, 25 µl, 100 µl, 150 µl, 200 µl, 20 µl, 25 µl, 30 µl, 36 µl, 72 µl, 144 µl, 15 µl, 37.5 µl, 50 µl, 25 µl, 50 µl, 75 µl, 100 µl, 150 µl and 200 µl respectively. Against *Sclerotium rolfsii* on potato dextrose agar (PDA) medium using Poisoned Food Technique (Nene and Thapliyal, 1982) [9].

The data presented in table 1, figure 1 and plate 1, revealed that among the six different fungicides and one antibiotics tested at different concentration, Difenconazole 25% EC, Hexaconazole 5% EC and Tebuconazole 25.9% EC were found highly effective at three different concentrations of each fungicides i.e. 15 µl, 20 µl, 25 µl, 100 µl, 150 µl, 200 µl, 36 µl, 72 µl, 144 µl respectively with 100 per cent inhibition of mycelial growth of S. rolfsii. Also, Propiconazole 25% EC and Thifluzamide 24% EC showed 100 per cent inhibition at concentration of (25 µl, 30 µl) and (50 µl, 75 µl) respectively. Whereas, Propiconazole 25% EC at concentration of 20 µl showed 94.44% inhibition of test pathogen. The other fungicides namely Kreoxime methyl 44.3% EC (11.11%, 22%, 83.3%) and Thifluzamide 24% SC (23%) were found to inhibit mycelial growth at concentrations of (15 µl, 37.5 µl, 50 µl) and 25 µl respectively. Among test antibiotics, Validamycin 3% L at concentration of 200 µl found least effective (11.11%) inhibition of *S. rolfsii* growth. Whereas, no inhibition was observed at concentration of 100 µl and 150 µl. In the present study, triazole fungicides group was found effective with strong antagonistic effect due to its strong anti-fungal property and it imparts the poisonous effect on pathogen's metabolic process, due to which adverse effect on growth of the *S. rolfsii* was observed.

The results of finding is in confirmation with the finding of earlier researchers, Banakar *et al*. (2017) [4] reported that fungicides like hexaconazole, tebuconazole, and combined products showed maximum inhibition of *S. rolfsii* at different concentrations used. Arunsari *et al*. (2011) [3] observed that triazoles fungicides *viz*., (hexaconazole, propiconazole, difenconazle) and its combined products containing traizoles were found to show inhibition to the mycelial growth of *S. rolfsii*.

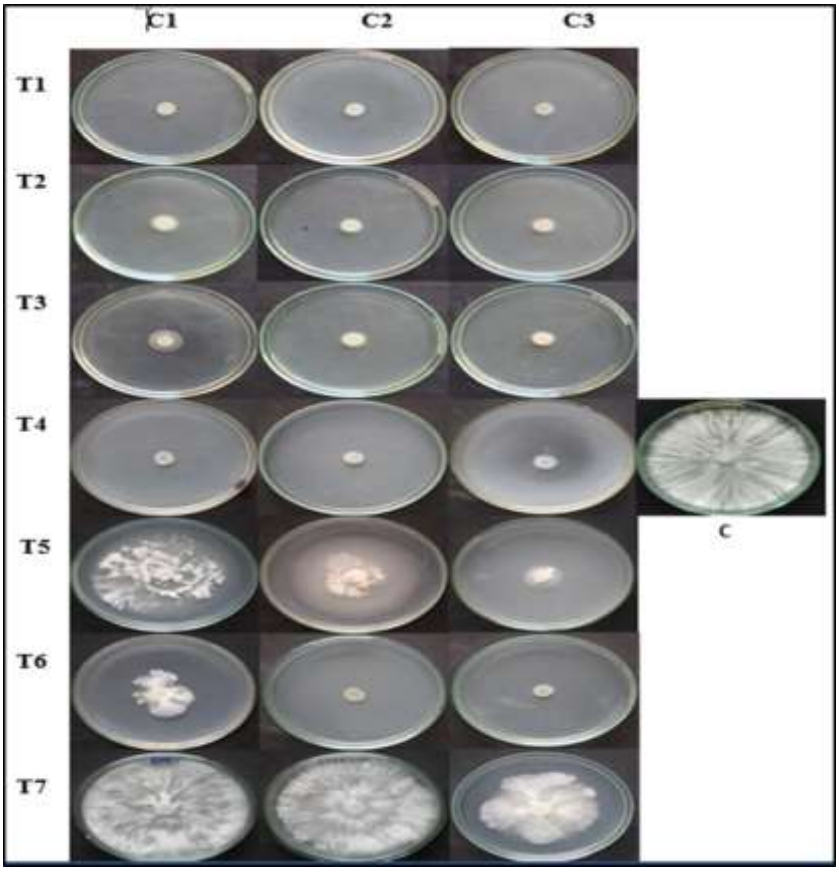

Plate 1: Antagonistic effect of different fungicides against *S. rolfsii*

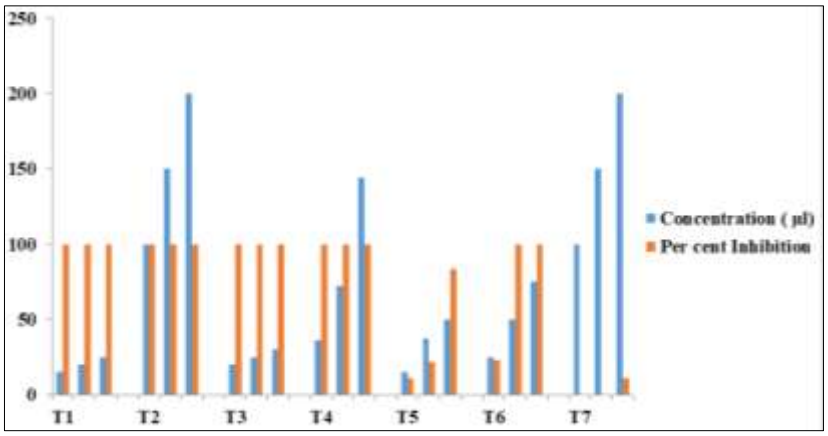

Fig 1: *In vitro* effect of fungicides against *Sclerotium rolfsii*

Table 1: *In vitro* evaluation of fungicides against *Sclerotium rolfsii*

Treatment	Fungicide	Conc. (µl)		Per cent inhibition
T_1	Difenconazole	C_1	15	100
		C_2	20	100
		C_3	25	100
T_2	Hexaconazole	C_1	100	100
		C_2	150	100
		C_3	200	100
T_3	Propiconazole	C_1	20	94.44
		C_2	25	100
		C_3	30	100
T_4	Tebuconzaole	C_1	36	100
		C_2	72	100
		C_3	144	100
T_5	Kroxime Methyl	C_1	15	11.11
		C_2	37.50	22
		C_3	50	83.30
T_6	Thifluzamide	C_1	25	23
		C_2	50	100
		C_3	75	100
T_7	Validamycin	C_1	100	0
		C_2	150	0
		C_3	200	11.11

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