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In vitro and *in vivo* Evaluation of Chemical Fungicides against *Sclerotium rolfsii* causing Collar Rot of Chickpea

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ABSTRACT: Collar rot of chickpea is well known disease in India with 2-5% of losses every year which may even reach up to 60% under severe conditions. Several chemical fungicides are widely available for controlling this disease and many chemicals have developed resistance against the disease. This study aims to cut down the cost of cultivation occurring due to use of non-effective chemicals and to provide hike in farmer's income. An experiment was conducted in laboratory and Agriculture Farm, BHU, Varanasi to assess the efficacy of some new fungicides *viz.* Tebuconazole (Folicur 250EC), Azoxystrobin (Onestar 23%SC), Flusilazole (Cursor 40%EC), Thiophanate Methyl + Kasugamycin (Kasu 44.8% + 2.6%) and Thiophanate Methyl (Roko 70%WP) against *Sclerotiumrolfsii*. These fungicides were prepared at two different concentrations *viz*. 100 and 300 ppm to evaluate their toxicity. Irrespective of the concentration, Flusilazole and Tebuconazole significantly inhibited the mycelial growth under *in vitro* conditions. All the fungicides were effective in controlling lesion diameter, Percent disease incidence, disease severity and in improving the pod yield. Among the tested fungicides, Flusilazole and Tebuconazole showed better performance under *in vitro* and *in vivo* conditions. These chemicals proved effective to be used on farmer's land to gain sustainable yields of 40.55 and 41.09% at higher concentration of Flusilazole and Tebuconazole.

Keywords: chemicals, fungicides, collar rot, chickpea, *Sclerotium rolfsii*

INTRODUCTION

Pulses (grain legumes) are the second most important group of crops worldwide. The major pulse crops those have been domesticated and are under cultivation are black gram, chickpea, cowpea, mung bean, lentil, moth bean, pea, pigeon pea etc. Among them, chickpea is widely cultivated and accounts to 75% of total pulse production of India (Ali *et al*., 2020). Singh *et al.* (1997) concluded that early record of Bengal gram in India is from 2000 BC in the parts of Uttar Pradesh and also many remnants of chickpea seed were excavated from Aurangabad from 300 BC to 100 BC. Chickpea is considered to be a healthy vegetarian food. Chickpea seed contains 29% protein, 59% carbohydrate, 3% fiber, 5% oil and 4% ash. Malic acid and oxalic acid from leaves are well known for their medicinal properties (Singh *et al*., 2020).

Chickpea is frequently subjected to various crop losses because of diseases and pests varying from 5-10% and 50-100% in temperate and sub-tropical regions (Kour *et al*., 2019; Singh *et al*., 2019). Widely distributed pathogens are *Ascochyta rabiei*, *Fusarium oxysporum* f.sp. *ciceri*, *Uromyces ciceris arietini*, *Razactonia bataticola*, *Sclerotium rolfsii*, Cucumber mosaic virus (Aswati and Math, 2020; Motagi *et al*., 2020). Among them, *Sclerotium rolfsii* is an important ubiquitous and polyphagous soil borne pathogen. It was first reported to be a causal organism of tomato blight from Florida by Rolfs (1892). Saccardo (1911) named the pathogen as *S. rolfsii*. High soil moisture coupled with optimum temperatures of 25-30°C, low soil pH and presence of debris near the surface of soil are highly suitable for the development of disease (Ghosh *et al*., 2013). Typical symptoms of chickpea are characterized by collar rot which occurs in wet soil conditions and seedlings collapse by turning yellow in color and older plants wilt. Affected roots are decayed and show rotting symptoms at collar region. Entire root is covered with white mycelial strands with mustard like sclerotia around the infected portion of root (Khan *et al*., 2020; Sharma *et al*., 2020). Several methods were followed to control this pathogen such as cultivating in disease free fields, altering the moisture levels, manipulating the date of sowing, soil solarization, biological control anduse of high resistant varieties. But chemical fungicides are found to be effective in controlling disease immediately compared to other management strategies (Ahsan *et al*., 2020). Based on necessity and performance, current experiment was designed in order to control *Sclerotium rolfsii* and improve the yield of chickpea in Uttar Pradesh.

MATERIALS AND METHODS

A. Isolation of pathogen

The root samples showing typical symptoms were collected from Agriculture Farm, BHU and packed in polythene bags and sealed. They were bought to laboratory for isolation of pathogen. Collected disease roots were first sterilized with ethyl alcohol using

cotton swab. Later they were cut into small pieces of 3 mm^2 size by using sterile scalpel. They are surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 30 sec. Then immediately rinse them in three changes of sterilized distilled water to remove the traces of mercuric chloride. Allow to air dry it by placing on sterilized filter paper and then transfer them on to PDA plated petri dishes using forceps. Inoculated plates were incubated in B.O.D incubator at 28 ± 2°C by providing favorable conditions for growth of pathogen. Cultures were purified by using hyphal tip method. It was done by picking up pure hyphal structure by using low power of the microscope and carefully transferring to fresh PDA petri dish and maintained at 25 ± 2℃ for 10 days. After purifying the infected fungus, their morphological and cultural characters such as color, size, growth rate, type of mycelium were recorded under microscope for their identity. By comparing with available standard literature, pathogen was identified as *Sclerotium rolfsii* (Barnett and Hunter, 1972).

B. In vitro evaluation of fungicides against *Sclerotium rolfsii*

Varying concentrations of newly marketed fungicides were used for *in vitro* and *in vivo* testing and many were found to be effective against *Sclerotium rolfsii.* Five fungicides were selected to test against *Sclerotium rolfsii* under laboratory conditions by following completely randomized design. Selected fungicides were Tebuconozole, Azoxystrobin, Flusilazole, Thiophanate + Kasugamycin and Thiophanate Methyl.

In the present *in vitro* experiment Table 1 fungicides were utilized against *Sclerotium rolfsii* by adopting poison food technique conducted in 2019.

Table 1: Amount of different fungicides used.

Sr. No.	Trade Name	Common Name	Percent a.i.	Company Name
1.	Folicur	Tebuconazole	250 EC	Bayer India
2.	Onestar	Azoxystrobin	23% SC	Dhanuka Agritech Ltd
3.	Cursor	Flusilazole	40% EC	Dhanuka Agritech Ltd
4.	Kasu	Thiophanate Methyl + Kasugamycin	44.8%+2.6%	Dhanuka Agritech Ltd
5.	Roko	Thiophanate Methyl	70% WP	Biostadt India Limited

Poison food technique was generally followed to assess the efficacy of above mentioned fungicides. Sterilized 80 ml potato dextrose agar medium was taken in 250 ml conical flask. Separately incorporate each fungicide aseptically on to PDA to 100 and 300 ppm concentrations. Then pour the medium in sterilized petri plates. Cut the disc of 5 mm diameter of *Sclerotium rolfsii* by using of sterilized cork borer from the 4 day old culture plates and transfer them on to petri plates. Place the disc in inverted position for the growth of fungus by incubating under favorable conditions of BOD at 27 ± 2ºC. Maintain a control devoid of fungicide. Each treatment is maintained under three replications. Measure the fungal colony diameter every day after inoculation with the help of scale recorded in mm. Calculate percent growth inhibition of each fungicide of various concentrations.

Preparation of fungicides solution for *in vitro* evaluation against *S. rolfsii.* All the fungicides were evaluated under two different concentrations of 100 and 300 ppm by poison food technique. Required concentrations of fungicides are obtained by adding appropriate amount of stock solution to PDA medium. M1V1 = M2V2, is used for calculating the fungicide concentration. Where, M1 is the concentration of the concentrated solution (stock solution), V1 is the volume of the concentrated solution (stock solution), M2 is the concentration of the dilute solution (after more solvent has been added), and V2 is the volume of the dilute solution. Just before the pouring, add a pinch of streptomycin sulphate and inoculate the fungus. Amount of chemical required to make 1 gm of a.i is given in Table 2.

Table 2: Amount of chemical required to make 1 gm of a.i.

Chemical Name	Amount of Fungicide used (for 1 gm a.i) in grams	Amount of distilled water (in ml)
Tebuconazole	3.68	100
Azoxystrobin	4.34	100
Flusilazole	2.50	100
Thiophanate + Kasugamycin	2.11	100
Thiophanate Methyl	1.42	100

Calculate the inhibition percentage of different fungicides over control with given this formula:

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

Where,

I = Per cent reduction in growth of *S. rolfsii*

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment

***In vivo* evaluation of fungicides against *Sclerotium rolfsii*.** Randomized block design was followed to evaluate the fungicidal efficiency under field conditions of chickpea during 2019. Size of the plot was 3m × 2m ($6m^2$) and the spacing was 30 cm × 10 cm. 45 days old chickpea plants were selected for inoculation. Fresh culture of four day old *Sclerotium rolfsii* was multiplied by sub culture technique and inoculated directly at the

portion of root near the ground region. Slight injury was made to root by using sterilized blade before inoculating fungus and covered immediately with moist cotton around it. Cover then root with soil and irrigate the field for the fungal growth. Observations are noted regarding the lesion developments at regular intervals.

Fungicides were sprayed after clear visible symptoms are seen on crop. Fungicides of two different concentrations *viz*., 100 and 300 ppm were prepared and sprayed on the crop using clean hand sprayer. Maintain three replications for each treatment and record the data at regular intervals by comparing with the control. Various morphological and yield parameters were recorded and analyzed during the period of experiment. Data was according to standard procedure.

RESULTS AND DISCUSSION

A. In vitro evaluation of chemical fungicides against *Sclerotium rolfsii*

Various fungicides were evaluated to control the toxicity of collar rot caused by *Sclerotium rolfsii* under *in vitro* conditions. Fungicides utilized were namely Tebuconozole (Folicur), Azoxystrobin (Onestar), Flusilazole (Cursor), Thiophanate Methyl + Kasugamycin and Thiophanate Methyl (Roko) under two different concentrations of 100 and 300 ppm. Food poison technique was used to figure out the effective fungicides against *S. rolfsii*.

Results regarding the control effect of fungicides are displayed in Table 3a. Among the tested fungicides Tebuconozole and Flusilazole were found to be very effective in controlling the pathogen. Complete inhibition was noticed with these fungicides at both the concentrations. This result was followed by Thiophanate Methyl + Kasugamycin fungicide which was also effective in controlling about 80% at 300 ppm concentration. Least suppression of pathogen was found with Azoxystrobin fungicide with only 65.92% inhibition rate at 300 ppm and 49.26% at 100 ppm concentration. So by this experiment, Tebuconozole and Flusilazole were concluded to be best fungicides even at lower concentrations and also Thiophanate Methyl controls maximum infection when used along with Kasugamycin compared to Thiophanate Methyl alone. The results obtained through this *in vitro* evaluation of fungicides found to be similar to the findings of Bhat and Srivastava (2003) where he found Thiophanate Methyl fungicide among the tested fungicides to be the best in controlling *S. rolfsii* at 250 ppm concentration (Fig. 1).

Table 3a: *In vitro* evaluation of different fungicides on mycelial growth of *Sclerotium rolfsii.*

Sr. No.	Fungicides	Radial growth (cm)		Percent Inhibition (%)	
		100 ppm	300 ppm	100 ppm	300 ppm
1	Azoxystrobin	4.56	3.06	49.26	65.92
2	Flusilazole	0.00	0.00	100	100
3	Thiophanate Methyl + Kasugamycin	0.78	0.50	91.29	94.37
4	Tebuconazole	0.00	0.00	100	100
5	Thiophanate Methyl	2.23	1.73	75.18	80.74
	Control	9.00	9.00	0.00	0.00
		Fungicide	**Concentration**	**Fungicide × Concentration**	
	SEm ±	0.111	0.044	0.221	
	CD at 5%	0.314	0.126	0.628	

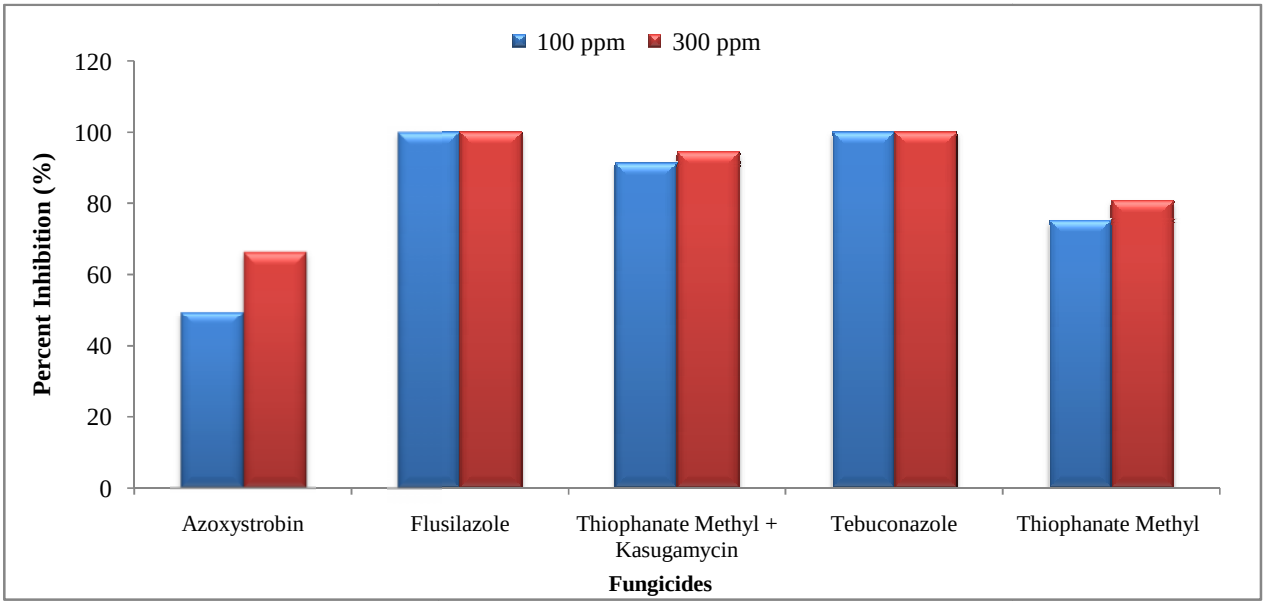

Fig. 1. *In vitro* evaluation of different fungicides on mycelial growth of *Sclerotium rolfsii.*

B. In vivo evaluation of different fungicides against Sclerotium rolfsii and its impact on yield

Similar fungicides (Tebuconozole (Folicur), Azoxystrobin (Onestar), Flusilazole (Cursor), Thiophanate Methyl (Roko) and Thiophanate Methyl + Kasugamycin (Kasu) were used at two different concentrations 100 and 300 ppm to determine their efficiency under field conditions. The results obtained were quite similar to that of lab conditions.

Various fungicides have shown variable disease severity ranging from 12–42% after spraying. Fig. 2a shows the effect of various fungicides on disease severity. Almost 100% of disease incidence was noticed in every case expect when crop was sprayed with Tebuconazole and Flusilazole where >80% of disease incidence is observed. Similarly disease severity ranged from 12.2 to 42.88%. This clearly depicts that Tebuconazole was found to be highly effective in controlling *Sclerotium rolfsii*.

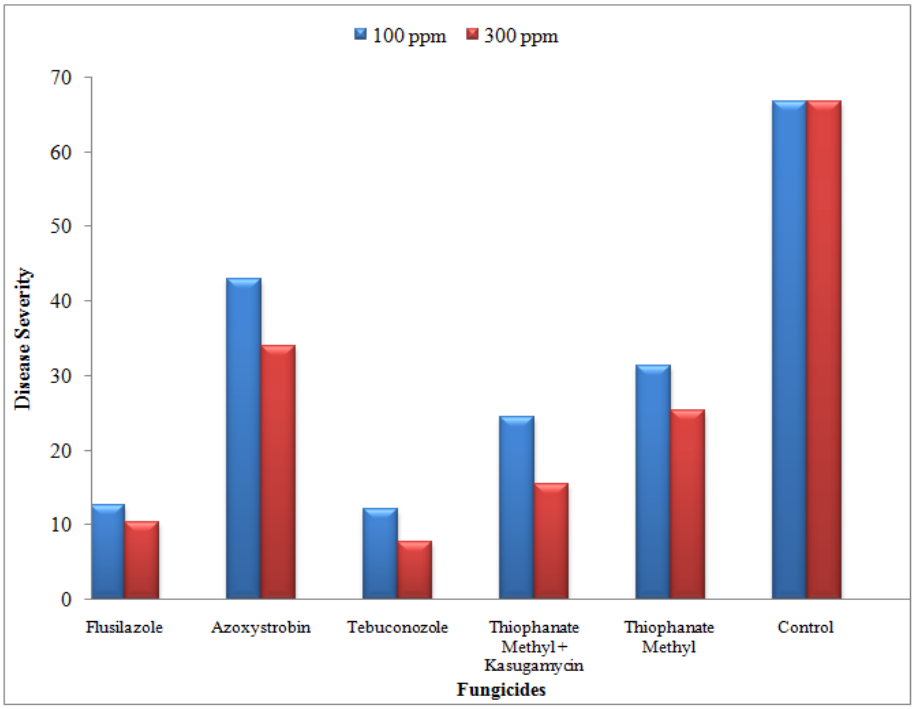

Fig. 2a. *In vivo* efficiency of different fungicides against *Sclerotium rolfsii* and their impact on disease severity.

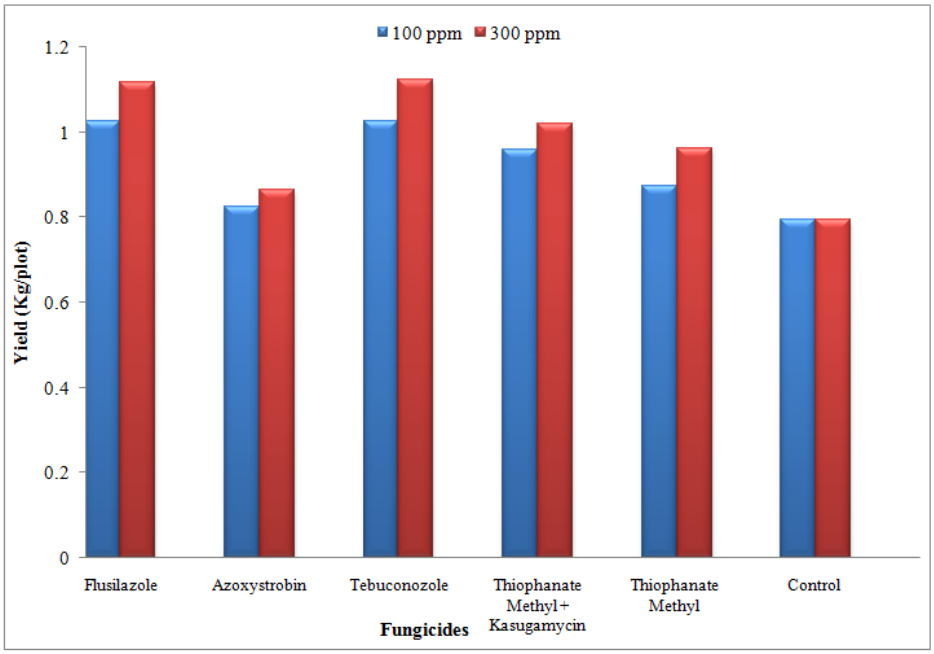

Fig. 2b. *In vivo* efficiency of different fungicides against *Sclerotium rolfsii* and their impact on yield.

This fungicide was followed by Flusilazole with almost similar disease severity. Azoxystrobin stood in last position in controlling the disease where almost 34% and 42.88% (300 and 100 ppm respectively) disease severity is observed. Thiophanate Methyl when combined with Kasugamycin showed less disease severity of 24.44% (100 ppm) and 15.55% (300 ppm) compared to Thiophanate Methyl alone with 31.33% (100 ppm) and 25.33% (300 ppm) disease severity. Similar trend was followed in case of number of pods and yield obtained after spraying the fungicides. Results (Table 3b) showed that yield was increased from 3.84 to 29% which recorded in producing 0.8 to 1.02 Kg/plot over 0.79 Kg/plot of control after treating with fungicides at 100 ppm.

Table 3b: Efficiency of different fungicides against *Sclerotium rolfsii* under field conditions and their impact on yield.

Sr. No.	Fungicides	Lesion diameter (cm)		Percent disease incidence		Disease severity		No. of pods/ plant		Yield (Kg/plot)		% Increase in yield	
		100 ppm	300 ppm	100 ppm	300 ppm	100 ppm	300 ppm	100 ppm	300 ppm	100 ppm	300 ppm	100 ppm	300 ppm
1	Flusilazole	1.90	1.56	86.66	80	12.66	10.44	48.00	52.33	1.025 (17.08 q/ha)	1.117 (18.62 q/ha)	28.92	40.55
2	Azoxystrobin	6.43	5.10	100	100	42.88	34.00	38.66	40.66	0.825 (13.76 q/ha)	0.863 (14.47 q/ha)	3.84	9.21
3	Tebuconozole	1.83	1.16	83.33	76.66	12.20	7.77	48.03	52.53	1.026 (17.09 q/ha)	1.122 (18.70 q/ha)	29.00	41.09
4	Thiophanate Methyl + Kasugamycin	3.66	2.33	100	100	24.44	15.55	44.83	47.70	0.957 (15.95 q/ha)	1.018 (16.98 q/ha)	20.41	28.12
5	Thiophanate Methyl	4.70	3.80	100	100	31.33	25.33	40.83	45.03	0.872 (14.53 q/ha)	0.961 (16.03 q/ha)	9.66	20.95
	Control	10.10	10.10	100	100	66.66	66.66	37.23	17.23	0.795 (13.25 q/ha)	0.795 (13.25 q/ha)		
SEm ±				0.197	0.149	0.200	0.266	0.668	0.948				
CD at 5%				0.643	0.486	0.651	0.869	2.180	3.092				

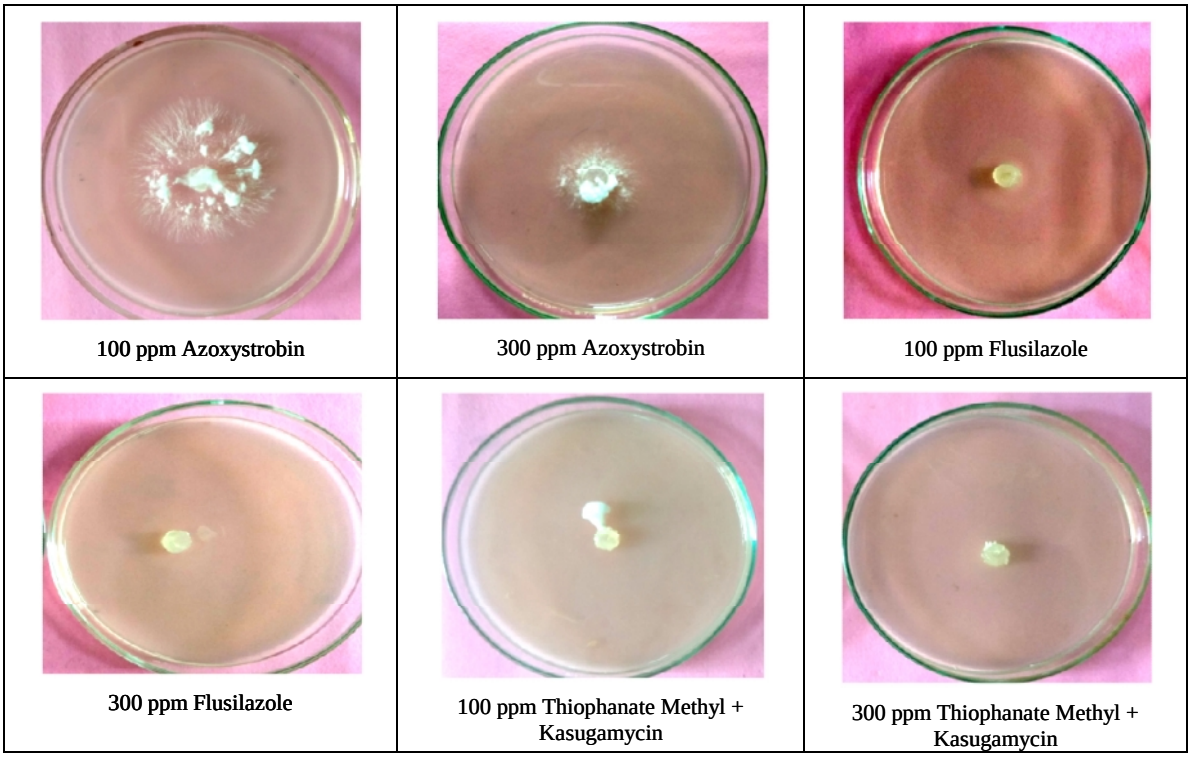

Plate 1. Effect of Azoxystrobin, Flusilazole and Thiophanate Methyl + Kasugamycin on mycelial growth of *Sclerotium rolfsii.*

Highest yield was reported to be from the plants treated with Tebuconazole and Flusilazole followed by Thiophanate Methyl + Kasugamycin and least with Azoxystrobin. Table 3 shows the yield obtained increased from 9.21 to 41.09% when sprayed with same fungicides at 300 ppm concentration with an average increase ranging from 0.86 to 1.11 Kg/plot. The obtained results were found to be similar with the findings of Vipin *et al*., (2011) where the variable efficiency of fungicides ranged from 20–25% with other fungal pathogens like *Rhizactonia solani*. Khan and Javaid (2015) reported the effectiveness of Thiophanate Methyl in reducing mortality % from 95 to 60% and Tebuconazole is found to be effective even when used at 50 ppm.

This current study concludes that Tebuconazole, Flusilazole and Thiophanate Methyl + Kasugamycin can effectively control *Sclerotium rolfsii* causing collar rot of chickpea under field conditions which thereby increase the yield comparatively.

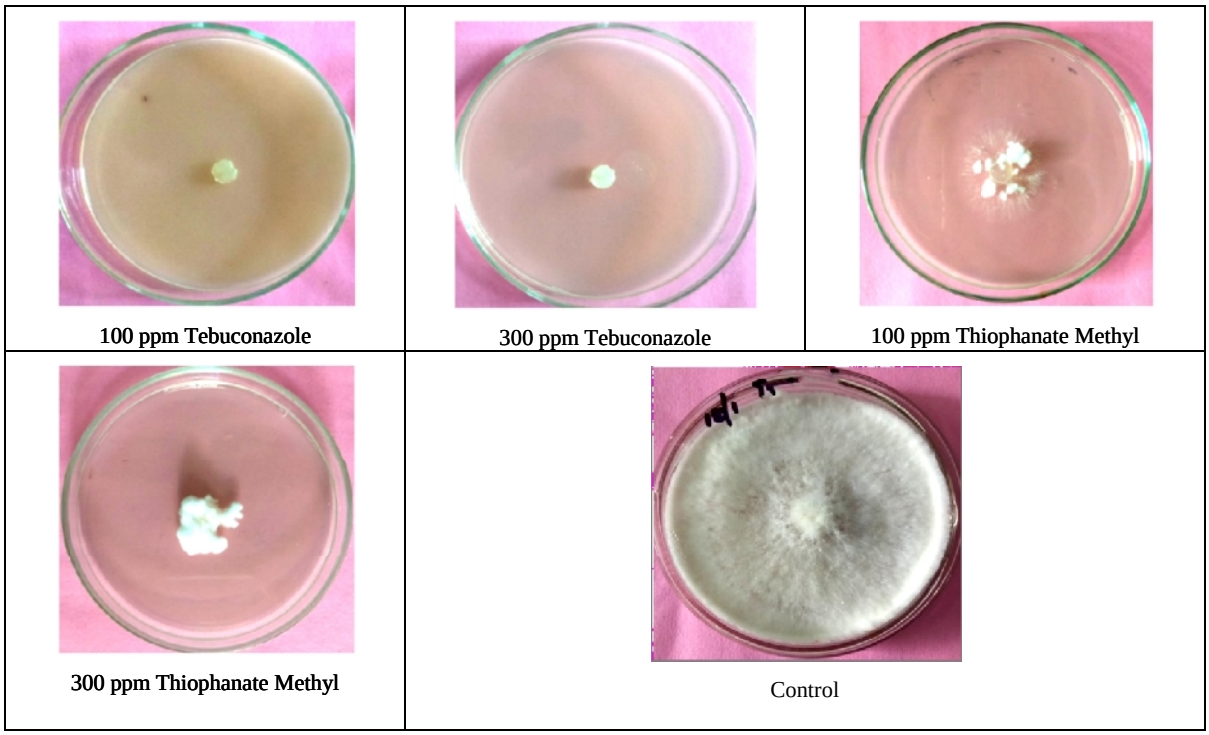

Plate 2. Effect of Azoxystrobin, Flusilazole and Thiophanate Methyl + Kasugamycin on mycelial growth of *Sclerotium rolfsii.*

SUMMARY, CONCLUSIONAND FUTURE SCOPE

All the tested fungicides were found to be more or less effective against the pathogen at 100 and 300 ppm concentrations under in vitro conditions. Complete inhibition of pathogen was found when treated with Flusilazole and Tebuconazole at all concentrations tested. Thiophanate Methyl + Kasugamycin have also reported about 91.29 and 94.37% at 100 and 300 ppm respectively. When Thiophanate Methyl was tested alone little decline in inhibition rate was noticed resulting in 80.74 at higher concentration. On the other hand, Azoxystrobin was reported to be least effective against pathogen.

Same fungicides were tested under field conditions against pathogen. The results obtained were co-related to that of in vitro conditions. Thus, Tebuconazole and Flusilazole treated plants have resulted with less disease severity of about 12% with 100 ppm concentration and below 7.77% with 300 ppm concentration. So, final yield obtained was much higher in these plants with 29% higher yield than control resulting about 1.02 Kg/plot with 100 ppm concentration and 1.12 Kg/ha under 300 ppm spraying. Thiophanate Methyl + Kasugamycin and Thiophanate Methyl also reported higher yield with 28.12 and 20.95% at higher concentration. Least increase in yield was observed from plants treated with Azoxystrobin with only 9.21% increase in yield under higher concentration.

Analyzing the effectiveness of novel chemicals available in market and using them in specific combination of bactericides can also greatly reduce the disease incidence. Utilizing specific concentration of particular chemical can cut the cost of cultivation. This study makes the farmer to choose efficient chemical to be used against collar rot disease of chickpea. This However, such research also improves provision of good quality fungicides. This prevents the exposure of farmers to fake produce sellers. This can also deduct the residual effect on soil and human health. In this way, chickpea growers can obtain higher yields by reducing the infection of *Sclerotium rolfsii* responsible for collar rot disease.

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