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Evaluation of Some Fungicides against Collar Rot Disease of Soybean

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ABSTRACT

A number of selected fungicides were evaluated to determine their efficacy for controlling collar rot disease of soybean plants caused by *Sclerotium rolfsii*. The experiment was conducted under the controlled condition at the Plant Pathology Laboratory and Field laboratory of BINA, Bangladesh Agricultural University campus from November 2018 to August, 2019. In-vitro research was done for the observation of radial mycelial growth of *S. rolfsii* on potato dextrose agar (PDA), treated with five fungicides viz. Antracol 70 WP (T1), Ridomil Gold MZ 68 WP (T2), Secure 600 WG (T3), Bavistin DF (T4), Dithane M-45 (T5) and one non-treated (T0) treatment. The highest percentage of mycelial growth inhibition of *S. rolfsii* in PDA medium was recorded in treatment T5 (Dithane M-45) 100% and lowest in treatment T3 (Secure 600 WG) 37.33% at 6 days after inoculation. Then the selected five fungicides were again applied to pot under controlled conditions to observe the best effect of selected fungicides against collar rot pathogen of soybean plants. The inoculation was done on a variety of BINA soybean 4 in pot condition. The highest mortality percent for the collar rot disease was found in treatment T0 (controlled) 100% soybean plants conversely, the lowest mortality percent was found in treatment T5 (Dithane M-45) 27.28% besides 38.92% in T2 (Ridomil Gold MZ 68 WP), 43.42% in T1 (Antracol 70 WP), 46.18% in T3 (Secure 600 WG) and 50.00% in treatment T4 (Bavistin DF) respectively. Thus, Dithane M-45 was found superior in controlling collar rot pathogen *S. rolfsii* of Soybean over all other fungicides tested in both *in vitro* and *in vivo*.

Keywords: Fungicide, Collar rot disease, *Sclerotium rolfsii*, Evaluation, and Soybean.

1. INTRODUCTION:

Soybean (*Glycine max L. Merr.*) is a leguminous crop and a good source of protein and vegetable oil. Soybean is providing 40% protein. It can play a vital role in balancing the protein deficiency of our diet (Mondal and Wahhab, 2001; Rahman, 2003). Soybean production of Bangladesh is 1.59 t/ha which is much lower than the world average (2.53 t/ha) (FAO, 2013). Soybean raw materials are used in different industries. Different soya foods are also prepared from soybean (Hossain *et al.*, 1992).

Soybean can fix to an extent of 300kg Nitrogen/ha/year and reduce the nitrogen requirement by 25-75% with *Bradyrhizobium japonicum*. Moreover, Soybean can fix atmospheric nitrogen symbiotically and therefore, through symbiosis about 80-90% nitrogen demand could be supplied by soybean (Keyser and Li, 1992). Thus this crop enhance soil fertility and economizes crop production not only for themselves but also for the next cereal crops and other non-legume crops grown in rotation and thereby, minimizing the regular rate of nitrogen fertilizer.

The production of soybean plants is low due to disease constrains. Soybean plants suffer from many diseases for example anthracnose, mosaic, collar rot, powdery mildew, etc. Out of them collar rot/ Foot and root rot of soybean is very severe damaging disease in Bangladesh, which is caused by *S. rolfisii*. It is common where high temperatures exist during the rainy season. The pathogen causes pre emergence and post emergence damping off, foot and root rot, collar rot and wilt of seedlings. Collar rot can occur in both seedling and adult plants. Collar rot /Foot and root rot disease is more common than southern blight or stem rot form in our country. Southern blight form of the diseases usually occurs on the older plants (Ahmed and Hossain, 1985). *S. rolfisii*, has the wide host range of more than 500 species of plants including many important crops such as maize, wheat, gram, khesari, lentil, mashkalai, mungbean, sunflower, sesame, brinjal, bitter gourd, bottle gourd, cowpea, cucumber, okra, radish, tomato, radish, chilli, coriander, garlic, onion, apple, peanut, soybean, and potato ; also many woody ornamentals, herbaceous annuals and perennials including ageratum, aucuba, azalea, begonia, columbine, coneflower, forsythia, hydrangea, marigold pansy, petunia, viburnum, and zinnia (Punja, 1985; Mullen, 2001).

Sclerotium rolfisii is both seed borne and soil borne pathogen (Fakir *et al.*, 1991). Collar rot causes yield loss of 10-25%, but under severe diseased conditions yield losses may ranges from 50 to 80% (Patil and Rane, 1982). It is very difficult to manage the pathogen because of its diverse nature of survival as large number of sclerotia produced and their ability to persist in the soil for several years. The fungus causes 25-50% loss through infection of seedlings. To control the fungus people usually use many chemicals. But the fungus *S. rolfisii* is a facultative saprophyte and can maintain continuity of its generation under adverse situation by the formation of sclerotia (Ahmed, 1980). Varietal resistance is another option for diseases management (Singh *et al.*, 2002). But there is no soybean resistant variety against collar rot in Bangladesh. So, it is necessary to search out effective fungicides to control this devastating disease. That's why the present research was taken under consideration to select the effective fungicides *in vitro* (Laboratory experiment) and *in vivo* (Pot Experiment) against collar rot disease of soybean caused by *S. rolfisii*.

2. MATERIALS AND METHODS:

The experiments were conducted during 2018 to 2019 in CRD design with thrice replication.

2.1 *In-vitro* evaluation (Laboratory Experiment) of different fungicides against *Sclerotium rolfisii*:

Poisoned food technique was used for evaluating the fungicidal effects on the growth of collar rot pathogen *S. rolfisii*. Five different fungicides viz., Antracol 70 WP (Propineb), Bavistin DF (Carbendazim), Dithane M-45 (Mancozeb), Secure 600 WG (Mancozeb and Fluazinam), Ridomil Gold MZ 68 WP (Mancozeb and Metalaxyl) were included in the experiment. The fungicides solutions were prepared in accordance with the labeled doses in the fungicide formulation. For example, preparation of 0.2% Antracol solution, 2 g of Antracol was taken into 1000 ml of sterilized distilled water. Poison food technique with little modification was followed for the evaluation of selected fungicides against *S. rolfisii*. Preparation of PDA containing fungicides was done in two steps.

In first step, clean peeled slice potato tubers of 125 g were boiled in 500 ml of water. Water was sieved from boiled potato slices and 10 g agar and 10 g Dextrose were added to it. PDA suspension was prepared up to 1000 ml of sterilized media before pouring in petridishes for preparing the original concentration. For preparation of poison food media 0.2g fungicide was mixed with 99.8 ml PDA media (Nene and Thapliyal, 1982). Isolation of the *S. rolfisii* was done by following standard tissue planting method from infected soybean plants. The infected tissues of soybean plants showing typical collar rot symptoms planted on potato dextrose agar (PDA) medium. The petriplates were incubated at 28±2°C were observed periodically for growth of the fungus. Pure culture of *S. rolfisii* was maintained on PDA slants for further studies. The pathogen was identified based on its mycelial and sclerotial characteristics (Sekhar *et al.*, 2017). The amended medium was then poured in sterilized Petri dish (90 mm dia.). A 5-7 mm disc of test fungus cut from the margins of ten days old cultures were placed centrally in each of the Petri dish and were incubated at 27°C. Mycelial growth colony diameter of *S. rolfisii* was measured at two days interval. It took 12 days to coverage of petridishes by mycelia. The percent mycelia growth inhibition (PI) of the fungus over control was calculated by using following

formula (Vincent, 1927). $PI = C-T/C \times 100$. Where, C= Growth of test pathogens in absence of fungicides (mm), T= Growth of test pathogens in presence of fungicides (mm). The diameter was measured with mm scale from the back side of the petridish taking it opposite to light.

2.2 In-vivo evaluation (Pot Experiment) of different fungicides against *Sclerotium rolfsii*: The *in vivo* evaluation of different fungicides was conducted in controlled conditions in the Field laboratory during April, 2019 to October, 2019. The experimental field was medium high land and the soil was sandy loamy. The experimental site belongs to Brahmaputra Alluvial Tract, the PH was 7.5. The soil of the experiment was collected from BINA farm field and then sun-dried, ground and screened through 10 mesh sieve to remove large particles and debris. The soil was dripped with 2% formalin solution 200ml per cft soil and kept covered with polythene sheets for 2-3 days. Then the soil was uncovered and kept for 4 days to release the gas formalin. Collected BINA soybean 4 seeds (10 seeds) were taken on a wet blotter paper in a petridish and kept for 4-7 days. Then the germination percent was 100% calculated by the formula of (Pankaj *et al.*, 2016):

$$\text{Germination (\%)} = \frac{\text{Number of Total Germinated Seeds}}{\text{Total Number of Seeds Tested}} \times 100.$$

Table 2: Different treatments along with mode of action, active ingredients, percent concentration and application rate (*in-vitro*).

| Treatments | Active ingredient | Mode of action | Application rate |
|--|--------------------------------|-------------------|------------------|
| T ₀ = Control | – | – | – |
| T ₁ = Antracol 70 WP | Propineb 70% | Contact | 2g/L |
| T ₂ = Ridomil Gold MZ 68 WP | Mancozeb 64% and Metalaxyl 8% | Systemic, Contact | 2g/L |
| T ₃ = Secure 600 WG | Mancozeb 50% and Fluazinam 40% | Contact | 1g/L |
| T ₄ = Bavistin DF | Carbendazim 50% | Systemic | 1g/L |
| T ₅ = Dithane M-45 | Mancozeb 80% | Systemic | 2g/L |

Seeds of soybean and required amount of individual selected fungicides (Provax) were taken in a petri-dish. Then few drops of sterilized water were added and shaken well with hands for few minutes and then kept open for drying. Then the seeds appeared to be coated with fungicides. Twenty-five seeds were sown in each pot on 28th June, 2019 and thinning was done at 15 DAS to maintain optimum plant

The seeds were treated with provax 200 0.2% before sowing. The soil was poured into perforated plastic pots at the rate of 11.50 kg/pot for 6 treatments including control. The following fertilizers (**Table 1**) were applied in each pot as per recommendation dose of the fertilizers Recommendation Guide (BARC, 2012) and total fertilizer were mixed uniformly and 5.22g/ pot were given two days before seed sowing.

Table 1: Fertilizers and recommended doses.

| Fertilizer | Doses (kg/ha) | Dose (g/pot) |
|---------------|---------------|--------------|
| Urea | 60 | 0.62 |
| TSP | 175 | 1.82 |
| MP | 120 | 1.25 |
| Gypsum | 115 | 1.20 |
| Zinc Sulphate | 6 | 0.062 |
| Boric acid | 10 | 0.104 |

The pots were tagged according to treatments received. Five fungicides (**Table 2**) were selected for pot experiment based on laboratory performance. Total six treatments were applied including control. There were 3 replications of each treatments and pots with fungicidal subsequent foliar spray.

population. At least 15-20 seedlings were kept for data collection. All the recommended agronomical and intercultural practices were strictly followed and the crop was given protective irrigation as and when necessary. Mass inoculums of *S. rolfsii* was prepared as described by (Giri *et al.*, 2003). In briefly, 500g lentil seeds were washed under sterilized water and taken into 1000 ml conical flask. Then pure culture

of *S. rolfisii* was inoculated and incubated for 12 days at room temperature and prepared 2% inoculums. Fungicidal spray was done on July 25, 2019 and *S. rolfisii* inoculum was sprayed on the collar region of each seedling 30 ml/pot on August 08, 2019. The experiment was monitored regularly to observe the first appearance of Collar rot. Spraying of fungicides was applied on twice sprays at weekly interval on July and August, 2019 by the sprayer (Siddique, 1997; Yaqub and shahzad, 2006). Mortality percent was assessed with proper formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead plants in a pot}}{\text{Total number of plants in pot}} \times 100.$$

2.3 Data Analysis - The data obtained from this research work were statistically analyzed following MSTAT-C program and treatment means in ANOVA tables were compared by LSD (0.05%) as followed by Gomez and Gomez (1984).

3. RESULT AND DISCUSSION:

The results were compiled based on the inhibition of radial mycelial growth and mortality percent of soybean plants. The collar rot symptoms of soybean plants showed mycelium covering the plant stem near the soil surface. The pathogen produced cottony mycelium with small brown sclerotia on the infected plant parts revealed the collar rot disease symptoms. Later, affected plants/branches turned yellow or drooped while retaining their green color, followed by drying and turning straw colored. White mycelial strands appeared at the collar region and above, covering the base of the branches. Whitish, brownish, irregular shaped sclerotia mingled with mycelial strands on branches. The similar findings

were described by Rekha *et al.* (2012), Kokub (2007), Prabhu and Patil (2004), Gupta and Sharma (2000), etc. The pathogen *S. rolfisii* was found whitish in colour in culture media and small mustard seed like sclerotia also found. The infected seedlings also showed cortical decay at the collar region and whitish mycelium was found extended on the soil surface. These findings were supported by Kulkarni *et al.* (1995), Aken and Dashiell, (1991), and Baruah *et al.* (1980).

3.1 In vitro evaluation (Lab experiment) - Efficacy of different treatments on radial mycelial growth of *S. rolfisii* was shown in **Table 3**. Fungicides have profound effect on reduction of radial mycelial growth of *S. rolfisii*. Here, it was observed that all the tested five fungicides significantly reduced the radial mycelial growth of *S. rolfisii* ranged from 0 to 45 mm recorded at 6 days after inoculation (DAI). The lowest radial mycelial growth (0 mm) of *S. rolfisii* was recorded in case of Dithane M-45 at 1, 2, 3 and 6 DAI. The lowest radial mycelial growth was observed in treatment T5 (Dithane M-45) 0 mm followed by treatment T4 (Bavistin DF) 15.8 mm, T1 (Antracol 70 WP) 16.5 mm, T2 (Ridomil Gold MZ 68 WP) 26.3 mm and T3 (Secure 600 WG) 28.2 mm after 6 DAI. Maximum radial mycelial growth (45 mm) was recorded in untreated T0 (Control) at 6 DAI. Again, the lowest radial mycelial growth of *S. rolfisii* was recorded in treatment T5 (Dithane M-45) 0 mm followed by treatment T4 (Bavistin DF) 22.2 mm, T1 (Antracol 70 WP) 23.9 mm, T3 (Secure 600 WG) 34.9 mm and T2 (Ridomil Gold MZ 68 WP) 35.5 mm after 10 DAI. The highest radial mycelial growth (45 mm) was recorded in untreated T0 (Control) again at 10 DAI.

Table 3: Evaluation of different fungicides against mycelial growth of *Sclerotium rolfisii* (*in vitro*).

| Treatments | Mycelial Growth of <i>Sclerotium rolfisii</i> on PDA Plate (mm) | | | | | | | |
|-----------------------|---|----------|----------|----------|----------|----------|----------|----------|
| | 02.04.19 | 03.04.19 | 04.04.19 | 07.04.19 | 08.04.19 | 09.04.19 | 10.04.19 | 11.04.19 |
| T ₀ | 10.60 a | 19.20 a | 28.90 a | 45.00 a | 45.00 a | 45.00 a | 45.00 a | 45.00 a |
| T ₁ | 7.50 bc | 10.70 c | 11.30 c | 16.50 c | 20.80 c | 21.00 c | 23.00 c | 23.90 c |
| T ₂ | 9.00 ab | 13.20 b | 16.60 b | 26.30 b | 29.20 b | 31.30 b | 33.80 b | 35.50 b |
| T ₃ | 6.60 c | 12.20 b | 17.80 b | 28.20 b | 29.80 b | 30.10 b | 34.80 b | 34.90 b |
| T ₄ | 6.16 c | 10.30 c | 11.70 c | 15.80 c | 19.20 c | 20.00 c | 21.90 c | 22.20 c |
| T ₅ | 0.00 d | 0.00 d | 0.00 d | 0.00 d | 0.00 d | 0.00 d | 0.00 d | 0.00 d |
| LSD _(0.05) | 1.72 | 1.41 | 1.99 | 3.22 | 2.31 | 2.99 | 3.13 | 4.24 |
| SE (±) | 0.559 | 0.459 | 0.648 | 1.04 | 0.750 | 0.969 | 1.02 | 1.38 |

** = Significant at 1% Level of Probability, * T₀ = Control, T₁ = Antracol 70 WP, T₂ = Ridomil Gold MZ 68 WP, T₃ = Secure 600 WG, T₄ = Bavistin DF and T₅ = Dithane M-45

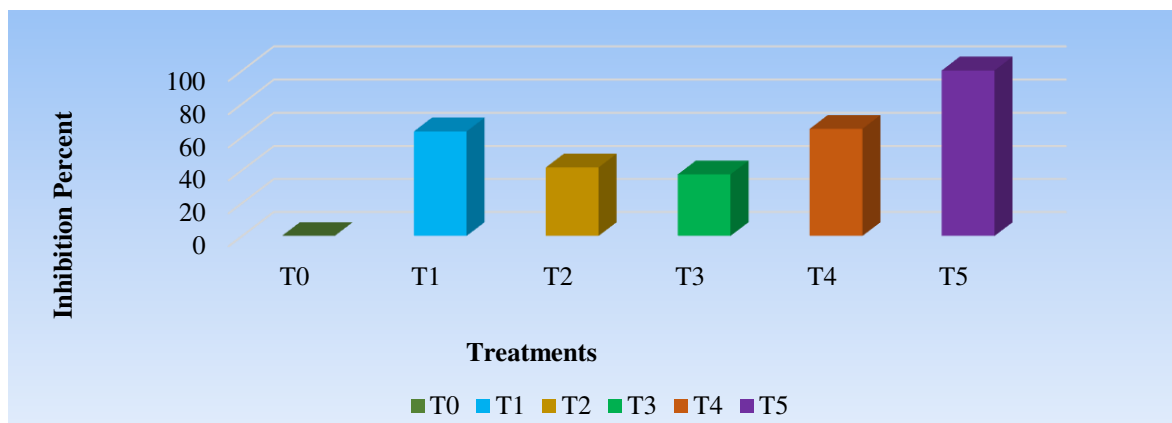


Fig 1: Effects of different fungicides on percent mycelial growth inhibition against *Sclerotium rolfsii* at 6 DAI (*in vitro*).

Here, T₀ = Control, T₁ = Antracol 70 WP, T₂ = Ridomil Gold MZ 68 WP, T₃ = Secure 600 WG, T₄ = Bavistin DF and T₅ = Dithane M-45. All the tested fungicides have strong effect on inhibition of mycelium growth against *S. rolfsii* in culture media. The highest percent of mycelial inhibition (100%) was recorded in case of treatment T₅ (Dithane M-45) followed by treatment T₄ (Bavistin DF) 64.88%, T₁ (Antracol 70 WP) 43.42%, T₂ (Ridomil Gold MZ 68 WP) 50% and T₃ (Secure 600 WG) 37.33% at 6 DAI (**Fig 1**).

Our findings were supported by Sangeetha and Jahagirdar (2013). They reported that Mancozeb (Dithane M-45), Carbendazim, (Carbendazim + Mancozeb) and (Carboxin + Mancozeb) were found to be effective in inhibiting mycelial growth of some pathogens including *S. rolfsii*. Among the tested five fungicides Dithane M-45 and Bavistin were also found to be toxic which caused 100% inhibition in growth of *S. rolfsii* at 0.2% concentration. These two fungicides were also recorded as fungicidal in action at the same concentrations. Antracol 70 WP, Secure 600 WG and Ridomil Gold MZ 68 WP have proved to be poor in this respect and did not show significant percent of inhibition and it was found by Chaurasia (2014). This result is in agreement with some researcher’s viz. Singh *et al.*, (2012), Sheoraj *et al.* (2005), Bhise (2002) and Harlapur (1988).

3.2 In-vivo evaluation (Pot experiment) - Data recorded on mortality percent of collar rot disease of

soybean plants as affected by the application of different fungicides were presented in **Table 4**. The effects of different treatments were recorded at 45 days after sowing (DAS) differed significantly as compared to control. The spraying of treatment T₅ (Dithane M-45) gave the lowest mortality percent 27.28%, T₄ (Bavistin DF) 38.92%, T₁ (Antracol 70 WP) 43.42%, T₃ (Secure 600 WG) 46.18% and T₂ (Ridomil Gold MZ 68 WP) 50%. The highest mortality percent was recorded in treatment T₀ (Control) treatment 100%. Among the treatments, T₅ (Dithane M-45) gave the best result for reducing percent mortality 27.28% of Soybean plants. Fungicides have profound effect on reduction of Mortality percent of soybean plants cultured in pot. All the tested five fungicides significantly reduced the mortality percent of the plants. The reduction of mortality percent of treatment T₅ (Dithane M-45) was 72.72%, T₄ (Bavistin DF) 61.08%, T₁ (Antracol 70 WP) 56.58%, T₃ (Secure 600 WG) 53.82%, T₂ (Ridomil Gold MZ 68 WP) 50% and T₀ (Control) 0% (**Table 4**). This data was obtained on November when temperature was 21°C and Relative humidity was 78%. This result is in agreement with some researchers viz. Mollah, (2012), Madhavi and Bhattiprolu (2011), Dasgupta and Maiti (2008); Johnson and Reddy (2008); Yaqub and Shahzad (2006), Tiwari and Singh (2004), Singh and Singh (2004), Siddique *et al.* (2002), Sugavanam *et al.* (1994), Kulkarni *et al.* (1994).

Table 4: Effect of different fungicides on mortality percent of soybean plants (in pot experiment).

| Treatments | Healthy Plants | Dead Plants | Mortality Percent (%) |
|----------------|----------------|-------------|-----------------------|
| T ₀ | 2.00 c | 2.00 cd | 100 |
| T ₁ | 7.67 b | 3.33 b | 43.42 |
| T ₂ | 2.00 c | 1.00 d | 50 |

| | | | |
|---------------------|---------|---------|-------|
| T ₃ | 4.33 c | 2.00 cd | 46.18 |
| T ₄ | 12.00 a | 4.67 a | 38.92 |
| T ₅ | 11.00 a | 3.00 bc | 27.28 |
| LSD _{0.05} | 2.37 | 1.18 | |
| SE (±) | 0.770 | 0.384 | |

Here, T₀ = Control, T₁ = Antracol 70 WP, T₂ = Ridomil Gold MZ 68 WP, T₃ = Secure 600 WG, T₄ = Bavistin DF and T₅ = Dithane M-45

On the other hand, Sharma and Ghosh (2017) reported collar rot disease yielded 55-95 % seedlings mortality of chickpea under heavy rainfall and high soil temperature (25-30°C). From the findings of the present experiment it may be concluded that treatment Dithane M-45 was most effective under *in-vitro* condition as it has completely inhibited mycelial growth of *S. rolfisii* up to 100% and exhibited minimum mortality percent of Soybean plants at 0.2% concentration. From this experiment, it was observed that Dithane M-45 reduced the growth of *S. rolfisii* significantly in *in-vitro* and reduced collar rot disease incidence of soybean *in vivo* condition.

4. CONCLUSION:

From the findings of the present experiment it may be concluded that Dithane M-45 was found completely inhibited the mycelial growth of collar rot pathogen *Sclerotium rolfisii* *in vitro* and exhibited minimum mortality percent of Soybean plants at 0.2% concentration. Again Dithane M-45 also showed superior performance in reducing collar rot incidence of soybean in pot experiment.

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6. CONFLICT OF INTERESTS:

The author (s) declared no potential conflicts of the interest with respect to the research, authorship and/or publication of this article.

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