

Bioactivity disruption of sclerotia through agrochemicals and temperature in *Rhizoctonia* Aerial Blight of soybean

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Rhizoctonia Aerial blight, caused by *Rhizoctonia solani* Kühn, is a significant global concern in soybean production. Numerous sclerotia of *R. solani* form in aerial blight-affected soybean parts and survive in the soil longer under adverse conditions. In the current study, we recorded the impact of fungicide and insecticide spraying on ten soybean genotypes on sclerotia formation in field conditions during Kharif 2022. The mean number of sclerotia per plant was significantly lowest (32.6) in plants sprayed with Tebuconazole 25.9% EC @ 1ml/litre (fungicide) than Thiamethoxam 12.6% + Lambda-cyhalothrin 9.5% ZC @ 0.25ml/litre (insecticide) (53.2) and water sprayed (68.9). In the laboratory, the germination of sclerotia was recorded after soaking it in 0.1 % agrochemicals, i.e. fungicides (three), insecticides (three) and herbicides (three), for the different periods 01, 24 and 72 hours in the PDA plate. Similarly, the influence of temperature (20, 30, 40 and 50°C for 3 and 6 hours) was also recorded on sclerotia germination. All three fungicides, i.e. Tebuconazole 25.9% EC, Tebuconazole 10% + Sulphur 65% WG and Carbendazim 12% + Mancozeb 63% WP, proved significantly effective in the reduction of sclerotia germination in all three duration of soaking. However, the best reduction was obtained in 72 hours of soaking (35.0 % germination) from Tebuconazole 10% + Sulphur 65% WG @ 0.1 % compared to sterile water (72.5 % germination). Insecticides and herbicides recorded no significant reduction in sclerotia germination. The study also indicated that continuous soaking of sclerotia, even in sterile water, also loses viability. Exposing the sclerotia to 40 and 50°C temperatures for 6 hours significantly reduced germination to 57.5 and 12.5 per cent, respectively. The findings of the present experiments might be helpful in the formation of strategies to reduce the primary inoculum of the aerial blight of soybeans in sensitive areas.

Keywords : Fungicides, germination, *Rhizoctonia solani*, sclerotia, soybean

INTRODUCTION

Soybean (*Glycine max* L.) is the main leguminous oilseed crop cultivated globally. It is also one of India's primary oilseed crops in the rainy season. However, India is among the top five soybean producers, but it still imports about 25% soybean oil to fulfil its local edible oil demand (Sagarika *et al.* 2023). Soybean has versatile uses, and products made from it are precious in terms of nutrition, which people in the lower-income class can afford (Uikey *et al.* 2022; Banerjee *et al.* 2023; Ramlal *et al.* 2023). Soybean

root nodule helps improve soil fertility by converting atmospheric nitrogen into a form that plants can use.

Due to all these attributes, soybean is called the "Golden Gift" of nature to mankind. Soybean is a major crop in central Indian states during the Kharif season. However, its cultivation faces challenges such as securing quality seeds, varying climatic conditions, inconsistent rainfall patterns, and the threat of diseases and pests (Agarwal *et al.* 2013; Rajput *et al.* 2021; Nataraj *et al.* 2023; Amrate, 2024; Amrate *et al.* 2024).

Plant disease is one of the major factors contributing to the low production of soybeans all over the world (Wrather *et al.* 2006; 2010;

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Bandara *et al.* 2020). Among these, *Rhizoctonia solani* Kuhn is one of the necrotrophic soil-borne fungi which can cause *Rhizoctonia* damping off and root rot and aerial blight in soybeans across the world (Wrather *et al.* 2010; Amrate *et al.* 2021). Aerial blight infects soybean crops in different parts of India, particularly in a more severe form in the states of Uttarakhand, Madhya Pradesh and Chhattisgarh (Wrather *et al.* 2010; Mathpal and Singh, 2017; Amrate *et al.* 2021). Aerial blight is a severe disease that infects several soybean genotypes and can cause up to 41 per cent yield losses (Amrate *et al.* 2018; 2023). Aerial blight exhibits water-soaked greyish brown lesions and mycelial web and sclerotial body of pathogen on affected plant parts of soybean (Fig.1). The development and progression of diseases are rapid under moderate temperatures and high humid field conditions (Amrate *et al.* 2021).

The *R. solani* is a taxonomically complex fungus with multinucleate cells that produce not conidia but sclerotia. This sclerotium is a compact form of aggregated melanized hyphae, which has a high survival ability even under stress conditions and survives in the soil. The abundant sclerotia form in the aerial blight affected plant parts of soybean during cropping season (Amrate *et al.* 2021). These sclerotia bodies play an essential role in the initiation and spread of disease. The severity of aerial blight may depend upon the number of sclerotial bodies present in the field or formed during the previous year's cropping season. In soybeans, the application of fungicides and insecticides is common during the reproductive stage of the crop. Agrochemicals might reduce sclerotia formation in aerial blight-affected soybean plants and may have an impact on sclerotia germination. More information is needed on the influence of agrochemicals on sclerotia formation and their germination and the influence of temperature on sclerotia survival, even in other crops. Therefore, the present investigation was undertaken to investigate the effect of foliar application of agrochemicals on sclerotial formation and to determine the effect of agrochemicals and different temperatures on sclerotial survival.

MATERIAL AND METHODS

Effect of spraying of agrochemicals on sclerotia formation

A field experiment was conducted to know the effect of spraying fungicide and insecticide on the formation of sclerotia (*R. solani*) on aerial blight-affected plants of soybean at J.N.K.V.V., Jabalpur (latitude 23°12'42"N and longitude 79°56'53"E) during Kharif season of 2022. The investigation was carried out on ten soybean varieties, namely JS 97-52, JS 20-29, JS 335, JS 20-94, JS 23-05, JSM-259, RVS 2001-04, RVS 2001-18, JS 20-34 and JS 22-05 sown in two rows plot of 3-meter row length in two replications. Recommended agronomic practices were adopted to grow the varieties across the season. The effect of spraying fungicide and insecticide on sclerotia formation was evaluated at 75 DAS (R-5 to R-6 stage). For this, ten plants were randomly selected, tagged and sprayed with Tebuconazole 25.9% EC @ 1ml/litre (fungicide), Thiamethoxam 12.6% + Lambda-cyhalothrin 9.5% ZC @ 0.25ml/litre (insecticide) and water separately. Sclerotia formed in fungicides, insecticides and water-sprayed plants were regularly monitored, and sclerotia were counted at 14 days after spraying from three trifoliate leaves (Amrate *et al.* 2021). An average number of sclerotia present in the plant were computed.

Effect of agrochemicals on sclerotia germination

A total nine agrochemicals viz., three fungicides (Tebuconazole 25.9%EC, Tebuconazole 10%+ Sulphur 65% WG, Carbendazim 12%+ Mencozeb 63% WP), three insecticides (Thiamethoxam 12.6%+ LambdaCyhalothrin 9.5% ZC, Emamectin Benzoate 1.9% EC, Chlorantraniliprole 18.50% SC) and three herbicides (Fluazifop-p-butyl 13.4% EC, Paraquat 24% SL, Glyphosate 41% SL) were tested at 0.1 % concentration against germination of sclerotia of *R. solani*. Freshly collected sclerotia (about one month old) (Fig. 1) were surfaced and sterilized in 1.0 per cent sodium hypochlorite for 1 min and then treated/ soaked in 0.1% concentration of the above agrochemicals for 1 hour, 24 hrs (1 day) and 72 hrs separately in glass vials. The treated sclerotia were picked,

dried in sterile filter paper in aseptic conditions for about 2 minutes, and placed on the sterile PDA medium. Ten such treated sclerotia were kept in a plate, and a total of four replications were kept for each set of treatments. The inoculated plates were incubated at $27\pm 1^{\circ}\text{C}$. The observation on germination of sclerotia by counting the mycelium colonies arising around the sclerotium was taken after two days of inoculation. The development of fungal growth around the inoculated sclerotia was considered a colony of those sclerotia. The percentage of sclerotia germinated was calculated using the following formula:

$$\text{Per cent sclerotia germinated} = \frac{\text{Number of sclerotia produced colony}}{\text{Total number of sclerotia inoculated}} \times 100$$

Effect of temperature on sclerotia viability

The sclerotia, used in the previous assay, were also utilized in the same study. Freshly collected sclerotia were kept at 20, 30, 40 and 50°C for 3 and 6 hours separately in sterile petri-plates. After temperature exposure of different durations, the sclerotia were surface sterilized and then inoculated in PDA plates (Thomidis *et al.* 2023). Four replications were kept for each treatment. All the inoculated plates were kept at $27\pm 1^{\circ}\text{C}$ temperature in an incubator for two days. After two days, the number of sclerotia germinated/produced colonies was recorded. The development of fungal growth around the inoculated sclerotia was considered as a colony of those sclerotia. The percentage of sclerotia germinated was calculated by using the formula given previously.

RESULTS AND DISCUSSION

Agrochemicals on sclerotia formation in field

A significant reduction in sclerotia formation was recorded in the case of fungicide and insecticide spraying (Table 1). The range of sclerotia among varieties was comparatively lower in the case of fungicide (23.0 to 39.6 per plant) and insecticide (41.1 to 66.5 per plant) than in water spraying (59.8 to 77.2 per plant). The mean number of sclerotia was significantly lowest (32.6 per plant) in plants sprayed with fungicide than insecticide (53.2 per plant) and water sprayed (68.9 per plant). These values revealed that spraying of

fungicide at 75 days reduced the sclerotia formation in sprayed plants. The significantly low formation of sclerotia in plants sprayed with fungicides may be due to a comparably lower aggregation of melanized hyphae and less hyphae development at plant parts. Previous studies also reported that fungicides like azoxystrobin and propiconazole affect mycelial growth and sclerotial production of *R. solani* (Xiang *et al.* 2023). Several studies show that fungicides inhibit the mycelial development of *R. solani* and reduce disease intensity in field conditions (Shailbala and Tripathi, 2004; Dutta and Kalha, 2011; Prakash *et al.* 2013). Another study showed that the application of fungicide significantly reduces the soil-borne inoculum of *Rhizoctonia solani* (Bartholomäus *et al.* 2017). Dung *et al.* (2018) also reported a reduction in germination of sclerotia of *Claviceps purpurea* in soil treated with fungicides. The present observation indicated that many survival structures were formed in all the varieties affected by aerial blight, which may lead to the next season's disease initiation. Hence, spraying fungicides may be beneficial as they reduce the inoculum formed in the affected plant. This may lead to low infection in subsequent hosts or following-season soybean crops.

Sclerotial germination affected by agrochemicals

In 1 hour of soaking/treatment, sclerotia germinated/colony produced ranged from 75.0 (Tebuconazole 10% + Sulphur 65% WG) to 95.0% (Sterile water). Only fungicides were significantly effective in reducing germination of sclerotia compared to sterile water dip. Other agrochemicals, including insecticide and herbicide, were non-significant in their efficacy. Similarly, in 24 hrs of soaking, sclerotia germination ranged from 65.0 (Tebuconazole 10% + Sulphur 65% WG) to 92.5% (Sterile water). Sclerotia germination was significantly reduced in a 72-hr soaking of agrochemicals. The germination percentage in 72-hour soaking ranged between 35.0 to 72.5%. The lowest germination was recorded by Tebuconazole 10% + Sulphur 65% WG (35.0%), followed by Tebuconazole 25.9% EC (40.0%) and Carbendazim 12% + Mancozeb 63% WP (45.0)

Table1: Effect of foliar application of fungicide and insecticide on *R. solani* sclerotia formation under field conditions at 14 days after application

Variety	Number of sclerotia/plant			Mean
	Water sprayed	Sprayed fungicide	Sprayed insecticide	
JS 97-52	65.2	32.1	58.3	51.8
JS 20-29	76.7	39.6	60.0	58.7
JS 335	76.8	38.5	66.5	60.6
JS 20-94	60.7	25.4	37.0	41.0
JS 23-05	65.2	33.5	52.6	50.4
JSM 259	70.0	35.0	53.7	52.9
PS 1660	77.2	38.5	62.1	59.3
RVSM 2011-35	75.2	35.0	56.6	55.6
JS 21-05	62.4	25.5	44.0	43.9
JS 20-69	59.8	23.0	41.1	41.3
Mean	68.9	32.6	53.2	
SE(m)+	A (Protection measures) = 1.2, B (Variety) = 2.2 A X B = 3.9			-
CD (p= 0.05)	A (Protection measures) = 3.5, B(Variety) = 6.5 A X B = NS			-

*Fungicide = Tebuconazole 25.9% EC@1 ml/Litre

Insecticide = Thiamethoxam (12.6%) + Lambda cyhalothrin (9.5%) ZC@0.25 ml/Litre

%). However, all three were significantly at par in their efficacy in reducing sclerotial germination. At the same time, other chemicals, including herbicides and insecticides, were found to be least effective even after soaking for 72 hrs. The germination percentage of sclerotia in all these chemicals was significantly close to sterile water (72.5). The study also indicated that continuous soaking of sclerotia, even in sterile water, also loses viability. This investigation revealed that only fungicides inhibited sclerotia germination, and herbicides and insecticides were ineffective. In previous research, Prakash *et al.* (2013) reported that carbendazim and hexaconazole were highly efficient in mycelial growth inhibition, sclerotial formation and sclerotial germination of *Rhizoctonia solani*, which are concurrent to our findings. They also indicated the partial efficacy of insecticides and herbicides in growth inhibition and sclerotia germination. In another study, sclerotia germination was entirely arrested by carbendazim at 10 µg/ml (Hemalatha, 2020). Xiang *et al.* (2023) reported that none of the fungicides inhibited the sclerotia germination of *R. solani*; however, in their study, a very short period of fungicidal soaked has been given. Similarly, our study also revealed that more

sclerotium germinated in 1 hour of soaking in fungicides. However, in contradiction to this, a report of herbicides such as Butachlor and Pretilachlor were found to be effective in inhibiting sclerotial germination (Sandhya *et al.* 2018).

Sclerotial germination affected by temperature

After 3 hrs of keeping the sclerotia at different temperatures, it was revealed that the sclerotia germination percentage was reduced to 57.5 % at 50°C. At the same time, it ranged from 85.0% (at 40°C) to 95.0 % (30 and 20°C) at other temperatures. Sclerotia germination per cent was statistically at par in cases 20, 30 and 40°C cases. However, it differed significantly at 40°C and 50°C (Table3). In the case of 6-hr keeping, the percentage of sclerotia germination was significantly reduced up to 12.5% at 50°C in comparison to 70.0% (40°C), 90.0% (30°C) and 95.0% (20°C) at different temperatures. The result revealed that sclerotia lost viability significantly at 50°C for 6 hours. In previous findings, it has been reported that *R. solani* was unable to grow at 5°C and 45°C, and sclerotia production was inhibited

Table 2: Per cent germination of *R. solani* sclerotia after soaking in 0.1% concentration of agrochemicals for different duration

Treatments	Agrochemicals	Percentage of sclerotia germinated after different period of soaking (Hours)		
		1	24	72
T1	Tebuconazole 25.9% EC	77.5(62.1)	67.5(55.2)	40.0(39.1)
T2	Tebuconazole 10% + Sulphur 65% WG	75.0(60.0)	65.0(53.7)	35.0(36.2)
T3	Carbendazim 12% + Mancozeb 63% WP	80.0(63.7)	70.0(56.9)	45.0(42.0)
T4	Thiamethoxam 12.6% + Lambda cyhalothrin 9.5% ZC	87.5(69.5)	80.0(63.4)	67.5(55.4)
T5	Emamectim benzoate 1.9% EC	85.0(67.4)	75.0(60.0)	65.0(53.7)
T6	Chlorantraniliprole 18.50% SC	87.5(69.5)	77.5(61.7)	70.0(56.9)
T7	Fluazifop-p-butyl 13.4% EC	90.0(71.5)	77.5(61.7)	60.0(50.8)
T8	Paraquat 24% SL	90.0(74.1)	80.0(63.7)	65.0(53.7)
T9	Glyphosate 41% SL	92.5(76.1)	82.5(65.4)	70.0(56.9)
T10	Sterile water	95.0(80.7)	92.5(76.1)	72.5(58.4)
	SE(m)+	3.46	2.3	2.2
	CD (p= 0.05)	10.0	6.8	6.4

The values in parenthesis are arc signed transformed

Table 3: Effect of temperature on *R. solani* sclerotia germination

Treatment	Temperature	Germination percentage	
		3 hours	6 hours
T1	20 ⁰ C	95.0(80.7)	95.0(80.7)
T2	30 ⁰ C	95.0(80.7)	90.0(74.1)
T3	40 ⁰ C	85.0(67.4)	70.0(56.9)
T4	50 ⁰ C	57.5(49.3)	12.5(17.8)
	SE(m)+	4.0	5.1
	CD (p= 0.05)	12.5	16.0

The values in parenthesis are arc signed transformed

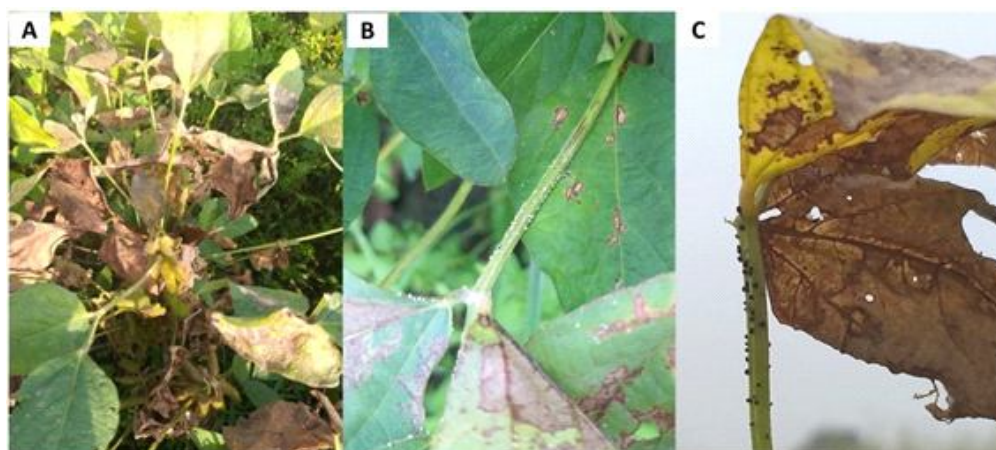


Fig.1: Soybean aerial blight affected plant (A) and initiation of sclerotia formation of *R. solani* (B) and presence of matured sclerotia on affected trifoliolate (C), respectively.

at 10 °C and its germination was optimum between 20-30°C (Ritchie *et al.* 2009). Hemalatha and Singh (2019) reported that *R. solani* sclerotia production was substantially better at 35°C, however, no mycelial growth from the sclerotia inoculated on PDA and kept at 40°C in the case of Banded Leaf and Sheath Blight of Maize. In our study, sclerotia germination was significantly reduced at 40 and 50°C temperatures. Similarly, exposure to sclerotia of *R. solani* (web blight of Urd bean) at 40°C reduces its survival rate from the beginning of storage and might be lost within nine months (Sharma and Tripathi, 2002). A study also reported that *R. solani*'s sclerotia causing pre-harvest fruit rot in tomato germinates ideally at 25 °C, is gradually reduced at 5°C and 35°C, and inhibited at 0°C and 40°C (Thomidis *et al.*, 2023). In our results, all the freshly collected sclerotia were viable. The viability of sclerotia can be observed even at 50°C (6 hrs). This indicates that the pathogen can remain viable under normal field conditions. However, the viability of sclerotia can be reduced by increasing the soil temperature through soil solarisation during the high temperature of the summer season. Our study also indicated that keeping sclerotia at 40°C for longer period may reduce their germination.

CONCLUSION

Numerous sclerotia of *R. solani* are formed in aerial blight-affected soybean parts. These sclerotia may serve as a primary source of inoculum for disease ignition in further seasons/ host. Spraying of recommended fungicides, i.e.

Tebuconazole 25.9% EC @ 1ml/litre, reduced sclerotia formation in aerial blight-affected plants. Among agrochemicals, fungicides only showed potential in reducing sclerotium germination, whereas herbicides and insecticides were impactless. Most of the sclerotia germinated at 20 and 30°C. However, the high temperature (40 and 50°C) hurt sclerotia germination. This information can be utilized to form effective disease management practices by reducing the load and potential of primary inoculum of aerial blight in soybeans.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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