

Short Communication

Effect of plant defense inducers on mycelial growth of *Sclerotinia sclerotiorum* causing stem rot of Indian mustard

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Abstract

Plant defense inducers are emerging compounds for disease management that can induce systemic resistance response in host plants at lower concentrations. Thus, the present study was undertaken to study the effect of plant defense inducers namely salicylic acid (SA) and sodium propionate (SP) on growth and development of *Sclerotinia sclerotiorum* which causes stem rot of Indian mustard. SA and SP along with carbendazim 50WP and azoxystrobin 23SC were tested against *S. sclerotiorum* using poisoned food technique at the concentration of 50, 100 and 150 ppm. The results showed that plant defense inducers with increased concentrations not only affected mycelial growth but also the formation of sclerotia, its weight and size significantly ($p=0.05$). Carbendazim 50WP at all tested concentrations was the most effective in completely inhibiting mycelial growth followed by SA @ 150 ppm (49.44%), and SA @ 100 ppm (30.59%). Although SP and azoxystrobin did not result in mycelial growth inhibition, they showed reduction in sclerotia count with increased concentrations. The lowest sclerotia count was observed for SA @ 150 ppm (12) while SP @ 50 ppm (54) showed the highest count. Inhibitory effect of chemicals on sclerotial growth subsequently reflected in reduction in weight and volume of sclerotia. Our findings show that SA and SP as plant defense inducers at tested concentrations possess lower antimicrobial activity as compared to fungicides and thus can be further utilized for disease management of stem rot of Indian mustard.

Keywords: Indian mustard, fungicides, plant defense inducers, *Sclerotinia sclerotiorum*

Introduction

Indian mustard is an important oilseed crop which possess high yield potential with 45% oil recovery ratio (Kumar, 2014). In India, the productivity of the crop is affected by various factors including diseases, and the stem rot of Indian mustard is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a major disease that affects quality and quantity of seed yield. The pathogen is a necrotroph having a wide host range which consists of more than 500 economically important cultivated plant species. The infection initiated at the flowering stage was reported to cause losses up to 100%. The high disease incidence of up to 66% and significant yield losses have been documented (Chattopadhyay *et al.*, 2015). The pathogen produces sclerotia which serve as overwintering structures and can remain viable up to 8-10 years (Adams *et al.*, 1979).

The unavailability of resistant variety and the lack of effective management practices lead to increased losses of seed yield. The cultural practices such as crop rotation, early sowing, adjusting plant density, scheduling irrigation and balanced application of fertilizers require continuous attention over longer periods of time. Although, preventive application of several fungicides including benzimidazoles, sterol

demethylation inhibitors, and dithiocarbamate have been found effective, these fungicides have not been registered for stem rot caused by *S. sclerotiorum* in many countries including India. Therefore, it is necessary to identify low-risk and more effective chemicals for sustainable management of *S. sclerotiorum*.

Plant defense inducers such as salicylic acid (SA) are emerging as an alternative to fungicides. These compounds can activate or prime plant immunity by mimicking the structure of natural immune inducers. They induce defense responses against a wide range of pathogens including fungi, bacteria, nematodes and viruses. These provide long term protection against a broad range of pathogens (Walters *et al.*, 2013). SA, an endogenous molecule involved in systemic acquired resistance has been found to induce expression of PR gene by leading to formation of reactive oxygen species (Klessig *et al.*, 2000) SA and its analogs have been widely studied for their ability to induce resistance against a wide range of pathogens. Sodium propionate (SP), an inorganic salt of propionic acid has been found to possess fungicidal properties and it is used in food industry as a preservative. The sodium propionate has been studied for its efficacy in controlling post-harvest diseases in stone-fruits (Biggs *et al.*, 1997; Blodgett *et al.*, 2002).

At present, no fungicides have been registered in India for management of stem rot disease caused by *S. sclerotiorum*. Therefore, the objective of the present study is to evaluate effect of plant defense inducers on mycelial and sclerotial growth of *S. sclerotiorum*.

Materials and Methods

Isolation of *Sclerotinia sclerotiorum*

The infected samples and sclerotia of Ludhiana isolate collected from research fields of Oilseeds section at Punjab Agricultural University, Ludhiana, were used for isolating the pathogen *Sclerotinia sclerotiorum* and the pure culture was obtained by hyphal tip culture method. The actively growing mycelium was used for *in vitro* and *in vivo* evaluation at different concentrations against plant defense inducers and fungicides.

Evaluation of antifungal properties of plant defense inducers

The evaluation of fungicides was carried out using poisoned food technique. The stock solution of 1000 ppm of salicylic acid, sodium propionate, carbendazim, and azoxystrobin was prepared. The desired amount of stock solution was amended to PDA media to obtain concentration of 50, 100 and 150 ppm respectively. The actively growing mycelial bit of 5 mm diameter was used for assessment. The un-amended PDA plate served as control. Three replicates for each treatment were maintained. The mycelial growth was obtained at 25°C. The radial growth was measured till control completes mycelial growth. The per cent growth inhibition was calculated using following formula:

$$\text{Percent growth inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in test plate}}{\text{Radial growth in control}} \times 100$$

Statistical analysis was performed two-way factor analysis for completely randomized design using OPSTAT software.

The effect on the sclerotia formation of *S. sclerotiorum* was also observed. The number of sclerotia formed per plate in each replication was recorded. The average weight of total sclerotia was recorded in grams. The length of the smallest and the largest sclerotium was measured which was further used to calculate the volume of sclerotium using the formula for volume of the cylinder. The average diameter of total sclerotia formed was taken into account to calculate the volume of the sclerotium. The effect of chemical on colony color and growth pattern were also recorded.

Results and Discussion

Plant defense inducers were tested at different concentrations along with fungicides using poisoned food technique. The results showed that with increased concentrations of plant defense inducers had a

significant effect on the radial growth inhibition of fungal growth (Table 1). The effect of defense inducers on the sclerotia formation, its number, average weight and volume were also examined which showed that with increased concentrations of plant defense inducers and fungicides had impact on sclerotial growth in terms of count, weight, size, and volume (Table 2). Average mycelial growth in control plate was 90 mm and yielded 44.5 mean count of sclerotia. The average weight of sclerotia in control was 0.4 g. The length of sclerotium was in the range of 1.4 to 4.5 mm while the volume was in the range of 1.6 to 5.1 mm³.

Carbendazim 50WP was the most effective in inhibiting complete mycelial growth at all tested concentrations suggesting that carbendazim is potential fungicide against *S. sclerotiorum*. It also inhibited the sclerotia formation. The systemic fungicide carbendazim belongs to benzimidazole group has been reported to affect tubulin integrity which is essential part of cytoskeleton of all fungal organisms (Davidse, 1986). The use of carbendazim at the concentration of 0.25 per cent as a foliar spray was found effective in stem rot suppression in Indian mustard (Kolte, 2005). Thus, carbendazim was found to be the most effective in inhibiting mycelial and sclerotial growth.

SA showed impact on mycelial growth with the increased concentration where the highest inhibition was shown by SA @ 150 ppm showing 49.4% inhibition of mycelial growth followed by SA @ 100 ppm and SA @ 50 ppm. SA also showed impact on sclerotial growth as well. Overall SA @ 150 ppm (12) resulted in the lowest count of sclerotia while SA @ 50 ppm (45) showed equal count of sclerotia as compared to control (44.5). Reduction in count of sclerotia also resulted in lowering of weight and volume. SA at tested concentration showed that range of weight of sclerotia was 0.2 to 0.6 g while volume was in the range of 1.1 to 7.9 mm³. The length of sclerotium was also impacted by SA at tested concentration which was observed in the range of 1 to 7 mm. Application of salicylic acid and its analogs have been reported to elicit resistance against several pathogens. Exogenous application of salicylic acid at the concentration 200 ppm found cent per cent inhibitory against stem rot of Indian mustard caused by *S. sclerotiorum* (Yadav *et al.*, 2017). The application of SA @ 200 ppm on cassava was the most efficient defense inducer which showed 33.3 per cent reduction in disease severity on anthracnose (Sangpueak *et al.*, 2021). Similar findings were reported for salicylic acid to suppress wilt of pepper caused by *Verticillium dahliae* Kleb. (Coşkun *et al.*, 2013), downy mildew of foxtail millet by *Sclerospora graminicola* (Hou *et al.*, 2023) and wheat leaf rust caused by *Puccinia triticina* (Elsharkawy *et al.*, 2022). This indicates that SA is effective in inhibiting the mycelial growth of *S. sclerotiorum*, with its efficacy

Table 1: Evaluation of different plant defense inducers against *Sclerotinia sclerotiorum* under *in vitro* conditions

Plant defense Inducer	Radial growth (mm)				Growth inhibition (%)			
	50 ppm	100 ppm	150 ppm	Mean	50 ppm	100 ppm	150 ppm	Mean
Salicylic acid	78.8	62.5	45.5	62.3	12.4	30.6	49.4	30.8
Azoxystrobin	90.0	90.0	90.0	90.0	00.0	00.0	00.0	00.0
Carbendazim	00.0	00.0	00.0	00.0	100.0	100.0	100.0	100.0
Sodium Propionate	90.0	90.0	90.0	90.0	00.0	00.0	00.0	00.0
Control	90.0	90.0	90.0	90.0	00.0	00.0	00.0	00.0
Mean	69.8	66.5	63.1		22.5	26.1	29.9	
	SEm±	CD (5%)						
Fungicide (F)	0.5	1.4						
Concentration (C)	0.5	1.1						
F×C	0.9	2.5						

Table 2: Effect of plant defense inducers on the growth of the *Sclerotinia sclerotiorum*

Chemicals	Concentration (ppm)	Sclerotia formation	Average no. of sclerotia	Average weight (gm)	Length of sclerotium (mm)		Volume of sclerotium (mm ³)		Colony color	Growth pattern
					Small	Large	Small	Large		
Salicylic acid	50	Yes	45	0.6	1.0	7.0	1.1	7.9	Greyish white	Irregular
	100	Yes	38.5	0.5	1.5	6.7	1.7	7.6	Greyish white	Irregular
	150	Yes	12	0.2	1.5	2.5	2.3	3.8	Greyish white	Irregular
Azoxystrobin	50	Yes	45	0.5	1.5	2.0	1.2	2.3	Greyish white	Uniform
	100	Yes	45	0.4	2.0	8.5	2.3	9.6	Greyish white	Uniform
	150	Yes	40	0.4	1.5	4.5	1.7	5.1	Greyish white	Uniform
Carbendazim	50	No	0	0.0	0.0	0.0	0.0	0.0	Nil	Nil
	100	No	0	0.0	0.0	0.0	0.0	0.0	Nil	Nil
	150	No	0	0.0	0.0	0.0	0.0	0.0	Nil	Nil
Sodium propionate	50	Yes	54	0.4	1.0	4.0	0.8	4.5	Greyish white	Uniform
	100	Yes	45.5	0.3	2.0	10.0	2.3	11.3	Greyish white	Uniform
	150	Yes	21.5	0.3	1.2	5.0	1.0	5.6	Greyish white	Uniform
Control		Yes	44.5	0.4	1.4	4.5	1.6	5.1	Cream white	Uniform

increasing with concentration.

Systemic fungicide, azoxystrobin, belongs to strobilurin group, has been found to interfere electron transport chain by binding at complex III in mitochondria and have been widely used for management of leaf spots and blight diseases caused by fungal plant pathogens (Oliver and Hewitt, 2014). However, Azoxystrobin showed no effect on the mycelial growth at all tested concentrations indicating zero percent inhibition. Treatment with azoxystrobin consistently resulted in greyish white colonies with uniform growth patterns across all concentrations. Azoxystrobin at tested concentrations yielded sclerotia count in the range of 40-45 weighing in the range of 0.4-0.5 g. However, azoxystrobin resulted in increased length and volume of sclerotia with the largest sclerotium of 8.5 mm in length and 9.61 mm³ in volume. It suggests that Azoxystrobin has variable impact on *S. sclerotiorum*.

Similar to azoxystrobin, sodium propionate showed no effect on the fungal growth at any concentration,

indicating zero percent inhibition. However, SP impacted sclerotial growth. SP @ 50 ppm (54) resulted in the highest count of sclerotia among all treatments. Also, SP @ 100 ppm yielded sclerotium of largest length (10 mm) and largest volume (11.3 mm³). However, SP showed lower average weight in the range of 0.3-0.4 g when compared to azoxystrobin. Sodium propionate showed a uniform growth at all concentrations tested. The propionic salt of sodium and calcium are used for preservation of fruits (Martin-Diana *et al.*, 2007). The propionic acid upon dissociation has been found to interfere with Krebs's cycle in mitochondria (Brock *et al.*, 2004). However, calcium and sodium might be regulating signaling pathways which aid in defense (Aghdam *et al.*, 2012). The preventive and curative application of sodium propionate was found effective in controlling soft rot of potato caused by *Pectobacterium* sp. (Yaganza *et al.*, 2014).

Statistically, the effect of plant defense inducers and fungicide and its concentration were observed to be highly significant. Thus, it could be inferred that

carbendazim is the most effective fungicide that resulted in complete growth inhibition of the pathogen even at lower concentrations. Overall results indicate that plant defense inducers and fungicides have variable impact on sclerotial growth of *S. sclerotiorum*.

Conclusion

This study unveils the antimicrobial properties of plant defense inducers against stem rot pathogen of Indian mustard, particularly at lower concentrations. Though salicylic acid and sodium propionate have lower antimicrobial activity at tested concentrations, these compounds exhibit a notable ability to lower disease progression by activating defense-related pathways within the host plant. Although plant defense inducers may fall short of the potency seen in the fungicide carbendazim, their effectiveness stands out, especially in addressing emerging strains resistant to traditional fungicides. This positions them as a promising alternative for future crop protection, emphasizing the need for a diversified and environmentally conscious approach in agriculture. As global agriculture faces challenges from evolving pathogens, there is the imperative to reduce reliance on conventional chemicals. Plant defense inducers offer a potential solution for disease management of stem rot in Indian mustard.

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