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To elicit systemic acquired resistance by chemical inducers against *Sclerotinia sclerotiorum*. (Lib.) de Bary

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Abstract
Stem rot or stem blight or white blight or white rot of Indian mustard incited by *Sclerotinia sclerotiorum (*Lib.) de Bary is an economically important yield reducing disease that has been widely reported in the last few years in India and elsewhere. Five systemic acquired resistance (SAR) activators in addition to carbendazim (standard check) were used against *Sclerotinia sclerotiorum* both *in vitro* and pot experiments. Salicylic acid was found cent per cent inhibitory at 200 ppm. while in pot conditions, azoxystrobin (26.00%) followed by salicylic acid (35.32%), as compared to control (52.20%). Maximum reduction in disease intensity over control was osberved with azoxystrobin (50.19%) followed by salicylic acid (32.34%) over control as seed soaking.

Keywords: Stem rot, *Sclerotinia sclerotiorum*, Indian mustard, SAR

Introduction
Indian mustard [*Brassica juncea* (L.) Czern & Coss] is one of the major oilseed crops in Rajasthan. The largest cultivation of *Brassica* crops is done for edible vegetable oil production. They also play a pivotal role in world's agricultural economy and are recognized for their long history of cultivation and varied uses. The oilseed *Brassica* crops are generally grouped as rape and mustard. Commonly cultivated species are *B. compestris* var. Sarson Prain, yellow and brown sarson; *B. campestris* var. toria; *B. juncea* Czern and Coss; rai and *Eruca sativa* Lam.: taramira. *Sclerotinia sclerotiorum* (Lib) de bary, the causal fungus of Sclerotinia rot or white blight or stem disease is a necrotrophic pathogen with world wide distribution known to infect over 400 species of plants (Boland and Halls, 1994) [4]. The pathogen affects many crops in India, particularly rapeseed and mustard and has become a wide spread and destructive in mustard growing parts (Ghasolia *et. al.,* 2004) [13] and take a heavy toll of yield (Chauhan *et. al.*, 1992) [5]. In mustard growing areas, this disease led to complete crop failure, as the disease incidence has been recorded up to 80 per cent in some parts of Punjab and Haryana states (Kang and Chahal, 2000; Sharma *et. al.* 2001) [15, 21]. Maximum incidence was recorded at Dausa (29.2%) district in Rajasthan followed by Rohtak (24.8%) districts in Haryana and minimum 7.0 and 7.4 per cent at Jaipur and Bharatpur districts, respectively (Yadav *et al.*, 2013) [24]. Once the pathogen is established, it is extremely difficult to control. This ascomycete can cause systemic and aerial infection by myceliogenic and carpogenic germination of *Sclerotinia* surviving in soil. Being ubiquitous necrotroph, it severely affects cultivated oilseed *Brassica* grown in different geographical regions of the world. However, fungicidal applications cause hazards to human health and increase environmental pollution. Induction of host resistance through chemical inducers and use of resistant cultivars are the effective solutions to overcome the problem.

Material and methods
Efficacy of SAR activators (*in vitro*)
The following systemic acquired resistance (SAR) activators in addition to carbendazim (standard check) were used.

Table 1: Different systemic acquired resistance activators

S. No.	SAR activators
1.	β-amino butyric acid
2.	Salicylic acid
3.	Hydrogen peroxide
4.	2,6-Di Chloroisonicotinc acid
5.	Azoxystrobin

The SAR activators (Table 1) in addition to carbendazim (standard check) were evaluated with three (100, 200 and 500 ppm) concentrations against the pathogen under laboratory conditions to find out their relative efficacy in inhibiting the growth of the pathogen in culture by poisoned food technique (Schmitz, 1930) [19]. Requisite quantity of each chemical was incorporated in sterilized two per cent potato dextrose agar medium, thoroughly mixed by shaking prior to pouring in sterilized Petriplates and were allowed to solidify. These Petriplates were inoculated with 5 mm disc of four day old culture in the centre of the plate and incubated at 25 $\pm$ 1^{0}C. Each treatment was replicated three with a suitable control. The efficacy of chemicals in each treatment and average of three replications was calculated. Per cent inhibition over control was calculated by the following formula (Bliss, 1934).

$$\text{Per cent inhibition over control} = \frac{C - T}{C} \times 100$$

C = growth of fungus in control
T = growth of fungus in treatment

Efficacy of SAR activators (*in vivo*)

The experiment was carried out in earthen pots (9 x 12 inches) with cultivar T-59 (Varuna). The pathogen multiplied on sorghum grains at 25 $\pm$ 1 ^{0}C for one week was used as the soil inoculum. Prior to sowing, pots were sterilized with copper sulphate solution and filled with sterilized soil (soil : vermicompost 3 :1). The soil was sterilized at 1.045 kg/cm^2 for one hour for three consecultive days. Varuna, the susceptible variety of Indian mustard was sown in these pots with four replications. The SAR activators viz., β-amino butyric acid (100 ppm), Salicylic acid (100 ppm), Hydrogen peroxide (100 ppm), 2,6-Di Chloroisonicotinc acid (100 ppm), Azoxystrobin (2000 ppm) and carbendazim (1000 ppm) were tested by applying as seed soaking (for 30 minutes), foliar spray (30 DAS) and seed soaking-cum-foliar spray.

These pots were inoculated with inoculum multiplied on sorghum grains 45 days after sowing. For inoculation the upper 5 cm layer of soil of each pot was thoroughly mixed with inoculum @ 20 g/pot. The pots were covered with polythene bags and kept for 24 hours in cage house.

To assess the stem rot intensity the following slightly modified disease rating (0-4) scale (Lesovoi *et al.*, 1987 and Sansford, 1995) [16] was followed.

Grade/numerical scale	Description/lesion lenghth on stem
0	Healthy (no visible lesion)
1	0.1-2.0 cm lesion length on stem
2	2.1-4.0 cm lesion length on stem
3	4.1-6.0 cm lesion length on stem
4	> 6 cm lesion length on stem or complete dried plant

The length of lesion on infected stem was considered for recording the disease intensity (Sharma, 1987). The infected area was calculated from 10 randomly selected plants at 80 DAS. In each pot and the average for each treatment was worked out. The intensity was calculated using the formula of Wheeler (1969) [23].

$$\text{Per cent disease intensity} = \frac{\text{Sum of individual ratings}}{\text{No. of plants observed x maximum disease rating}} \times 100$$

Results and discussions

Efficacy of SAR activators (*in vitro*)

The efficacy of SAR activators in addition to carbendazim was evaluated against *Sclerotinia sclerotiorum* on PDA by poisoned food technique. The data suggested (Table 1) that increase in concentration of the SAR activators caused increased inhibition of mycelial growth of the fungus. Among these, salicylic acid was found cent per cent inhibitory at 200 ppm. This was followed by β-amino butyric acid (77.77, 86.66 and 92.00 %) at 100, 200 and 500 ppm, respectively. Azoxystrobin (18.48, 50.22 and 73.33%) and 2, 6-Dichloroisonicotinic acid (24.44, 38.52 and 80.00%) were found to be least effective at 100, 200 and 500 ppm, respectively against *Sclerotinia sclerotiorum*. All the SAR activators (β-amino butyric acid, salicylic acid, hydrogen peroxide, 2, 6 Dichloroiso nicotinic acid and azoxystrobin) in addition to carbendazim were tested at 100, 200 and 500 ppm concentration and inhibited mycelial growth of *S. sclerotiorum.* Our observations are in agreement with El. Ganaieny *et al.* (2002) [10], Shahda (2002) [20], Hilal *et al.* (2006) [14], Abdel-Monaim *et al.* (2012) [1] and Rahaman *et al.* (2012) [18]. They reported that all the tested abiotic agents were found significantly superior in reducing linear growth of many fungal pathogens including *S. sclerotiorum*.

Efficacy of SAR activators (*in vivo*)

Seed soaking

A perusal of data (Table 3) revealed minimum disease intensity with azoxystrobin (26.00%) followed by salicylic acid (35.32%), as compared to control (52.20%). Maximum reduction in disease intensity over control was osberved with azoxystrobin (50.19%) followed by salicylic acid (32.34%) over control. β-amino butyric acid (28.93%) was found at par with salicylic acid. Minimum reduction in disease intensity was observed in 2, 6-Dichloroisonicotnic acid (22.22%).

Foliar spray

Perusal of data (Table 3) revealed a similar trend of results as in 4.3.1. The highest reduction in disease intensity over control was observed in azoxystrobin (60.73%) followed by

salicylic acid (49.82%), β-amino butyric acid (45.45%), hydrogen peroxide (38.73%) and minimum in 2, 6 Dichloroiso-nicotinic acid (34.55%).

Seed-cum-foliar spray

A perusal of data (Table 3) revealed minimum disease intensity in azoxystrobin (14.65%) followed by salicylic acid (21.90%) and β-amino butyric acid (23.90%) over control (48.12%).

Maximum reduction in disease intensity over control was observed in azoxystrobin (69.56%) followed by salicylic acid (54.49%), β-amino butyric acid (50.33%) and minimum in 2, 6 Dichloroiso nicotinic acid (42.02%).

Protection of plants against pathogens depends on constitutive and induced defense mechanisms (Nurnberger and Lipka, 2005) [17]. Extensive research has shown that biotic and abiotic agents including salicylic acid play a key role in local and systemic acquired resistance (SAR) to biotrophic and necrotrophic pathogens (Durrant and Dong, 2004; Gaffney *et al.,* 1993; Thomma *et al.,* 1998 and Conarth *et al.,* 2002) [8, 12, 22, 6]. Amongst different SAR activators used in the present investigation were applied through seed soaking, foliar and seed-cum-foliar spray. In these methods, seed soaking-cum-foliar spray of SAR activators was found most effective to control disease by reducing disease intensity, followed by foliar spray and seed soaking alone. All SAR activators tested were able to reduce the disease intensity significantly over control. Azoxystrobin, a fungicide-cum-SAR inducer was the most effective in reducing the disease intensity followed by salicylic acid, β-amino butyric acid, hydrogen peroxide and 2, 6 Dichloroisonicotinc acid. These results are in agreement with the results of Dantre *et al.* (2003) [7], Abdel-Monaim *et al.* (2012) [1], El Bana (2007) [9], El-Mougy *et al.* (2004) [11] and Shalaby *et al.* (2001). They reported effectiveness of various SAR inducer in disease control against many fungal pathogens including *S. sclerotiorum*.

Table 2: Efficacy of SAR activators against *Sclerotinia sclerotiorum* by poisoned food technique after 7 days of incubation at 25 ± 1 ^{0}C

SAR activators	Per cent growth inhibition at various concentration (ppm)			
	100	200	500	Mean
β-amino butyric acid (BABA)	77.77 (61.87)	86.66 (68.58)	92.00 (73.57)	88.14 (68.01)
Salicylic acid	90.37 (71.92)	100.00 (90.00)	100.00 (90.00)	96.79 (83.93)
Hydrogen peroxide	16.66 (24.09)	38.88 (38.57)	100.00 (90.00)	51.85 (50.89)
2,6-Di Chloroisonicotinic acid	24.44 (29.63)	38.52 (38.36)	80.00 (63.43)	35.43 (43.81)
Azoxystrobin	18.48 (25.46)	50.22 (45.13)	73.33 (58.91)	47.34 (43.17)
Carbendazim (Standard check)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Control	0.00	0.00	0.00	0.00
			SEm±	CD (p=0.05)
		A	1.00	2.87
		C	0.66	1.88
		AxC	1.74	4.97

*Average of three replications

Figures given in parenthesis are angular transformed value

Table 3: *In vivo* efficacy of SAR activators against *Sclerotinia* rot of Indian mustard

SAR activators	Concentration used (ppm)	Seed soaking		Foliar spray		Seed soaking-cum- foliar spray	
		Disease intensity (%)*	Per cent disease control	Disease intensity (%)*	Per cent disease control	Disease intensity (%)*	Per cent disease control
β-amino butyric acid	100	37.10 (37.52)	28.93	30.00 (33.21)	45.45	23.90 (29.27)	50.33
Salicylic acid	100	35.32 (36.46)	32.34	27.60 (31.69)	49.82	21.90 (27.90)	54.49
Hydrogen peroxide	100	39.60 (39.00)	24.14	33.70 (35.49)	38.73	27.00 (31.31)	43.89
2,6-Di Chloroisonicotinic acid	100	40.60 (39.58)	22.22	36.00 (36.87)	34.55	27.90 (31.88)	42.02
Azoxystrobin	2000	26.00 (30.66)	50.19	21.60 (27.69)	60.73	14.65 (22.50)	69.56
Carbendazim (Standard check)	1000	10.82 (19.20)	79.27	12.65 (20.83)	77.00	6.32 (14.56)	86.87
Control	-	52.20 (46.26)	0.00	55.00 (47.87)	0.00	48.12 (43.92)	0.00
SEm±		0.69	-	0.87	-	0.41	-
CD (p=0.05)		2.19	-	2.77	-	1.31	-

*Average of four replications

Figures given in parenthesis are angular transformed value

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