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Management strategies for Alternaria leaf spot of soybean (*Glycine max*) caused by *Alternaria alternata*

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ABSTRACT

Alternaria leaf spot disease caused by *Alternaria alternata* is one of the most economically important disease in soybean production. Efficacy of four fungicides and two botanical formulations were tested against six isolates of *A. alternata* collected from major soybean [*Glycine max* (L.) Merr] growing areas of Rajasthan during rainy (*kharif*) season 2018 and 2019 at Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur. Among the fungicides tested, Azoxystrobin 8.3% + Mancozeb 66.7% WG was the most effective fungicide that caused 100% inhibition of mycelial growth of all the six isolates of *A. alternata* followed by Azoxystrobin 23% SC at 500 and 1000 ppm, and neem oil at 0.5% was found most effective *in vitro*. In field condition most virulent isolate (UDP Aa-1) were used for artificial inoculation with spore suspension having concentration 1×10^3 conidia/ml on the plants of 45 DAS. Among the ten treatments, combination of Azoxystrobin 8.3% + Mancozeb 66.7% WG at 0.36% + Neem oil at 0.5% was found most effective in reducing the disease intensity and increase seed yield followed by individual applications of Azoxystrobin 8.3% + Mancozeb 66.7% WG at 0.36% over untreated control. These results suggest that botanical extract with fungicides has a great potential to control the leaf spot disease of soybean in eco-friendly way.

Keywords: *Alternaria alternata*, Disease intensity, Fungicides, Leaf spot, Soybean

Soybean [*Glycine max* (L.) Merr] is one of the most important oilseed crops. It has a prominent place among modern agricultural commodities, as it is the world's most important seed legume which contributes 25% to the global edible oil (Pagano and Miransari 2016). Soybean contains highest protein (40–42%) among cultivated pulses. It also contains 18–20% oil content with calcium, iron and glycine and is used as protein concentrates, vegetable oil for humans and a high-quality animal feed (Fagodiya *et al.* 2022). Among different constraints in soybean production, the most serious are diseases like anthracnose, bacterial blight, bacterial pustule, brown spot, charcoal rot, frog eye leaf spot, *Fusarium* root rot, pod and stem blight, purple seed stain and *Cercospora* leaf blight, *Rhizoctonia* aerial blight, Sclerotium blight, rust, virus and seedling diseases that have been reported in India (Wrather *et al.* 2006).

In India, Alternaria leaf spot disease of soybean caused by *A. alternata* was reported by Shrivastava and Gupta (2001). Alternaria leaf spot of soybean is common in Illinois during the latter part of the growing season (Chamberlain 2011). On foliage, the disease symptoms develop as brown necrotic spots with concentric rings and yellow halo, large necrotic lesions that eventually coalesce and consume the entire leaf in advanced stages (Fagodiya *et al.* 2021). The disease infected seeds are small, shrivelled and characterized by dark irregular spreading sunken area (Bhosale *et al*. 2014). Disease incidences up to 30% of *A. alternata* infecting soybean have been reported from Turkey and infected plants displayed necrotic, circular to oval, and dark brown spots on the upper surfaces of the lower leaves (Ustun *et al.* 2019). Application of botanicals is a sustainable approach apart from being a promising alternative to fungicide application. Thus, the present study was aimed to evaluate two botanicals and four fungicides *in vitro* and to determine optimal timing of their application for the control of Alternaria leaf spot on soybean.

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MATERIALS AND METHODS

Cultures of *A. alternata* were isolated from the severely infected plants from farmers' fields during *kharif* 2018. Based on cultural and morphological characteristics, the isolates were identified as *A. alternata.* Further confirmation was done by identifying it from Indian Type Culture Collection (ITCC) and Division of Plant Pathology, IARI, New Delhi. (ITCC ID No. 10.810.18, 2018). The pure culture was transferred on PDA slants and maintained for further studies.

In vitro *evaluation of different fungicides and plant* *extracts:* Efficacy of four fungicides (Azoxystrobin 8.3% + Mancozeb 66.7% WG, Azoxystrobin 23% SC, Mancozeb 75% WP and Pyraclostrobin 20% WG) at (500 and 1000 ppm) and two botanicals, viz. Azadirachtin and Neem oil were evaluated at 0.5% against *A. alternata* using poisoned food technique during 2018 at Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur. Sterilized fungicide amended PDA with desired concentration was poured in sterilized (90 mm) glass petri plates, allowed to solidify (five replications). A suitable untreated control was also maintained. Each petri plate was inoculated with active growing 5 mm mycelial discs of pathogen and incubated at 27±1ºC temperature for 7 days with factorial randomized design. Colony diameter (two diagonals) was measured at 24 h after interval and continued till the untreated control plate was fully covered with mycelial growth of the test fungus. Per cent inhibition of mycelial growth was calculated by using formula given by Bliss (1934).

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

where I, Per cent inhibition; C, Colony diameter in control; T, Colony diameter in treatment.

Effect of foliar spray with fungicides, botanicals and their treatment combinations on Alternaria leaf spot of soybean in field condition: The experiment was carried out at Instructional Farm, Rajasthan College of Agriculture in randomized block design (RBD) with three replications using susceptible cultivar (RKS-24) of soybean during *kharif* season 2018 and 2019. The foliar sprays of the fungicides, plant extracts and their treatment combinations which were found most effective *in vitro* were done alone and also in combination with neem oil that was the most effective botanical *in vitro* against *A. alternata.* Solutions of Azoxystrobin 8.3% + Mancozeb 66.7% WG (0.24%, 0.30%, 0.36%), Azoxystrobin 23% SC (0.1%), Mancozeb 75% WP (0.4%), Pyraclostrobin 20% WG (0.1%), Azadirachtin (0.5%) and Neem oil (0.5%) were prepared alone and in combination, thereby making ten treatments in all, these were then sprayed twice i.e. first after 24 h of inoculation and second at 15 days interval. Artificial spray inoculations were made by making an inoculum of *A. alternata* with spore suspension having concentration 1×10^3 conidia/ml. For comparison, inoculated control was maintained without fungicidal/botanical application. Observations of PDI were recorded at 15 days interval upto maturity stage. Per cent disease index was calculated with help of disease rating scale (0–5) given by Sangeetha and Siddaramaiah (2007). The details of the scale are as follows: 0 = Leaves free from infection, 1 = Small irregular spots covering <5% leaf area, 2 = Small irregular brown spots with concentric rings covering 5.1–10% leaf area, 3 = Lesions enlarge, irregular brown with concentric rings covering 10.1–25% leaf area, 4 = Lesions coalesce to form irregular and appears as a typical leaf spotting symptom covering 25.1–50% leaf area and 5 = Lesions coalesce to form irregular and appears as a typical leaf spotting symptom covering >50% leaf area.

The per cent disease intensity (PDI) and per cent efficacy of disease control (PEDC) were calculated by using following formula given by Wheeler (1969).

$$\text{PDI} = \frac{n \times 1 + n \times 2 + n \times 3 + n \times 4 + n \times 5}{B} \times \frac{100}{\text{Maximum disease score (5)}}$$

where n, number of plants in each score; 1-5 = disease score; N, total number of plant under observation

$$\text{PEDC} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

RESULTS AND DISCUSSION

Efficacy of fungicides and botanicals on radial growth of A. alternata*:* A perusal of data (Table 1) revealed that all test fungicides at 500 and 1000 ppm and botanicals at 0.5% significantly inhibited the radial growth of six test isolates of *A. alternata* over control, but the isolates exhibited variable sensitivity to the fungicides and botanicals.

The combined product Azoxystrobin 8.3% + Mancozeb 66.7% WG was the most effective fungicide at 500 and 1000 ppm inhibited 100% radial growth of all the six isolates of *A. alternata* and the isolates exhibited variable response to application of individual fungicide. At 500 ppm, the maximum growth inhibition among isolates of *A. alternata* was observed to be with UDP Aa-1 (81.8%) followed by PRA Aa-1 (80.0%) and minimum growth inhibition was recorded with BAR Aa-1 (71.5%). Pyraclostrobin 20% WG was found least effective and the maximum inhibition among isolates of *A. alternata* was observed by UDP Aa-1 (67.0%) followed by PRA Aa-1 (68.8%) and minimum growth inhibition was observed to be JHA Aa-1 (61.8%). At 1000 ppm the maximum inhibition among isolates of *A. alternata* was observed by UDP Aa-1 (86.2%) followed by PRA Aa-1 (83.3%) and the minimum growth inhibition was recorded with BAR Aa-1 (75.3%). Pyraclostrobin 20% WG found least effective showed that maximum inhibition among isolates of *A. alternata* was observed with UDP Aa-1 (73.1%) followed by PRA Aa-1 (71.6%) and minimum growth inhibition was observed by BAR Aa-1 (63.7%). Neem oil was observed to be significantly superior in inhibiting the radial growth of the six representative isolates. Among the isolates Neem oil caused, maximum inhibition of isolate UDP Aa-1 (64.1%) of *A. alternata*, followed by CHI Aa-1 (61.4%) and minimum growth inhibition was observed with BAR Aa-1 (51.8%) followed by Azadirachtin showed that maximum inhibition of isolate UDP Aa-1 (52.22%) of *A. alternata*, followed by CHI Aa-1 (50.1%) and least growth inhibition was observed with BAR Aa-1 (39.7%). The way in which fungal populations (isolates) respond to fungicides used for their control and hence the risk of disease control failures due to resistance, depends primarily on the type of genetic variability available. Existing evidence indicates that the type of variability differs with the chemical rather than with the organism and the same

Table 1 *In vitro* efficacy of various fungicides and botanicals against six isolates of *A. alternata* from respective districts

Isolate	Per cent growth inhibition* at 500 ppm					Per cent growth inhibition* at 1000 ppm				Per cent growth inhibition* at 0.5%	
	Control	Azoxystrobin 8.3% + Mancozeb 66.7% WG	Azoxystrobin 23% SC	Mancozeb 75% WP	Pyraclostrobin 20% WG	Azoxystrobin 8.3% + Mancozeb 66.7% WG	Azoxystrobin 23% SC	Mancozeb 75% WP	Pyraclostrobin 20% WG	Azadirachtin	Neem oil
UDP Aa-1	0.0	100.0 (90.0)	81.8 (64.8)	72.7 (58.5)	67.0 (54.94)	100.0 (90.0)	86.2 (68.2)	77.5 (61.7)	73.1 (58.7)	52.22 (46.2)	64.1 (53.2)
CHI Aa-1	0.0	100.0 (90.0)	78.1 (62.1)	69.6 (56.5)	65.1 (53.8)	100.0 (90.0)	81.6 (64.6)	75.0 (60.0)	69.3 (56.3)	50.1 (45.0)	61.4 (51.6)
PRA Aa-1	0.0	100.0 (90.0)	80.0 (63.4)	71.6 (57.8)	68.8 (56.1)	100.0 (90.0)	83.3 (65.9)	76.7 (61.1)	71.6 (57.8)	48.6 (44.2)	60.8 (51.2)
KOT Aa-1	0.0	100.0 (90.0)	75.1 (60.0)	67.8 (55.4)	62.7 (52.3)	100.0 (90.0)	79.0 (62.7)	73.0 (58.7)	67.2 (55.0)	41.7 (40.1)	54.4 (47.5)
BAR Aa-1	0.0	100.0 (90.0)	71.5 (57.7)	64.4 (53.3)	58.6 (49.9)	100.0 (90.0)	75.3 (60.2)	69.1 (56.2)	63.7 (53.0)	39.7 (38.9)	51.8 (45.9)
JHA Aa-1	0.0	100.0 (90.0)	74.7 (59.7)	67.1 (54.9)	61.8 (51.7)	100.0 (90.0)	78.8 (62.6)	71.4 (57.7)	66.5 (54.6)	44.7 (42.0)	57.6 (49.3)
		CD at 5%	CD at 1%	CD at 5%	CD at 1%	CD at 5%	CD at 1%	CD at 5%	CD at 1%	CD at 5%	CD at 1%
	Fungicide	1.01	1.37	0.78	1.05	0.40	0.54	0.45	0.60	1.57	2.08
	Isolate	1.11	1.50	0.85	1.15	0.44	0.59	0.49	0.66	2.03	2.69
	F/B X I	2.49	3.35	1.91	2.58	0.99	1.34	1.10	1.48	3.51	4.67

*Mean of five replications; Figures in parentheses are arcsine √per cent angular transformed values.

phenomenon is observed in the present study that different isolates responded variably to variously tested fungicides. All the isolates were found to be highly sensitive against the combined product of Azoxystrobin and Mancozeb rather than with their individual application; it is basically because the combined product is mixture of systemic and contact fungicides and so its mode of action is broad-spectrum. In accordance with the current results by Zade *et al.* (2018) reported that Hexaconazole, Propiconazole, Propineb and Cymoxonil + Mancozeb showed 100% mycelial growth inhibition of *A. alternata* in soybean. Bhosale *et al.* (2014) reported that garlic extract exhibited significant reduction against *A. alternata* in soybean. Madadi *et al.* (2021) reported Flutriafol 6.94% + Tebuconazole 20.8% caused complete mycelial growth inhibition of *A. alternata.* Our results are also collaborated with (Nivedha *et al.* 2019) and (Balai *et al.* 2020) for mycelial growth inhibition against *A. alternata.*

Effect of foliar spray with fungicides, botanicals and their treatment combinations on Alternaria leaf spot of soybean in field condition: Out of 10 treatments, combination of Azoxystrobin 8.3% + Mancozeb 66.7% WG at 0.36% with neem oil at 0.5% was found most effective in reducing disease with PDI 11.6%, higher PEDC with 82.7%, having maximum yield 1787.49 kg/ha and higher yield increase 82.9% followed by sole application of Azoxystrobin 8.3% + Mancozeb 66.7% WG at 0.36% with PDI (16.9%), PEDC (74.8%), yield (1707.86 kg/ha) and yield increase (74.7%) as compared to control. The individual applications of Azoxystrobin 23% SC with PDI (31.4%), PEDC (53.3%), yield (1467.12 kg/ha) and yield increase with 50.1%. However, the individual application of botanicals resulted in higher PDI as compared to chemicals, neem oil sprayed plots exhibited PDI (48.3%), PEDC (28.2%), yield (1224.96 kg/ha) and yield increase (25.3%). Maximum PDI 67.3% and lowest yield was recorded in control (Table 2). Combination of fungicides along with botanical like neem oil not only helps in reducing the hazardous effects rendered to the environment but also improves the vitality of the plants. This combination is having a high degree of synergistic effects as the chemical has a broad-spectrum antifungal mode of action and application of neem oil renders plant to a very high degree of resistance against the pathogen as it is having anti-fungal secondary metabolites like terpenoids. The results of the study also concur with the finding of Kgatle *et al.* (2020). The Epoxyconazole + Pyraclostrobin and Azoxystrobin + Difenoconazole were the most effective spray fungicides in reducing Alternaria leaf blight in sunflower. Balai *et al.* (2020) reported the combination of Mancozeb with *H. Rufa* was found most effective in reducing Alternaria blight of pigeon pea. Furthermore, many studies were conducted (Rajput and Chaudhari 2018 and Adhikar and Roy 2019) to understand the effects of combination product on the growth and severity of *A. alternata*.

Chemical management is most favorable and widely

Table 2 Efficacy of fungicides and botanicals on severity of Alternaria leaf spot and its effect on yield of soybean

Treatment	PDI after 30 days of inoculation*			Per cent efficacy of disease control (PEDC)**			Yield (kg/ha)			Per cent yield increase		
	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled
Azoxystrobin 8.3% + Mancozeb 66.7% WG 0.24%	24.2	26.4	25.3	63.4	61.4	62.4	1589.81	1512.03	1550.92	58.7	58.5	58.6
	(29.4)	(30.9)	(30.1)	(52.8)	(51.6)	(52.2)				(50.0)	(49.9)	(49.9)
Azoxystrobin 8.3% + Mancozeb 66.7% WG 0.30%	19.6	20.2	19.9	70.3	70.5	70.4	1664.81	1598.14	1631.47	66.2	67.5	66.8
	(26.2)	(26.7)	(26.4)	(57.0)	(57.1)	(57.0)				(54.5)	(55.3)	(54.9)
Azoxystrobin 8.3% + Mancozeb 66.7% WG 0.36%	16.5	17.3	16.9	75.0	74.7	74.8	1737.03	1678.70	1707.86	73.4	76.1	74.7
	(23.9)	(24.5)	(24.2)	(60.0)	(59.8)	(59.9)				(58.9)	(60.8)	(59.8)
Azoxystrobin 23% SC 0.1%	30.6	32.2	31.4	53.7	52.9	53.3	1508.33	1425.92	1467.12	50.6	49.7	50.1
	(33.5)	(34.5)	(34.0)	(47.6)	(46.7)	(46.9)				(45.3)	(44.8)	(45.1)
Mancozeb 75% WP 0.4%	36.1	37.3	36.7	45.4	45.3	45.4	1433.33	1340.74	1387.03	43.1	40.7	41.9
	(36.8)	(37.6)	(37.2)	(42.3)	(42.4)	(42.3)				(41.0)	(39.6)	(40.3)
Pyraclostrobin 20% WG 0.1%	41.2	43.5	42.3	37.7	36.4	37.1	1358.33	1254.62	1306.47	35.6	31.58	33.6
	(39.9)	(41.2)	(40.6)	(37.9)	(37.1)	(37.5)				(36.6)	(34.1)	(35.4)
Azadirachtin 0.5%	53.2	55.7	54.4	19.6	18.6	19.1	1204.62	1081.48	1143.05	20.2	13.4	16.8
	(46.8)	(48.2)	(47.5)	(26.2)	(25.5)	(25.9)				(26.7)	(21.4)	(24.1)
Neem oil 0.5%	47.4	49.3	48.3	28.3	25.8	28.2	1282.40	1167.52	1224.96	28.0	22.5	25.3
	(43.51)	(44.6)	(44.0)	(32.1)	(31.6)	(31.8)				(31.9)	(28.2)	(30.1)
Azoxystrobin 8.3% + Mancozeb 66.7% WG 0.36% + Neem oil 0.5%	10.4	12.9	11.6	84.2	81.1	82.7	1811.11	1763.88	1787.49	80.8	85.1	82.9
	(18.8)	(21.0)	(19.9)	(66.6)	(64.2)	(65.4)				(64.0)	(67.4)	(65.7)
Control	66.2	68.5	67.3				1001.85	953.70	977.77			
	(54.4)	(55.8)	(55.1)									
CD (P=0.05)	3.95	4.28	2.43	6.38	6.38	3.65	75.27	83.39	46.96	46.96	7.52	4.00
CV%	6.68	6.88	6.78	7.92	7.92	7.61	3.01	3.53	3.26	3.26	10.29	9.46

*Mean of three replications, figures in parenthesis are √arcsine percent angular transformed values; **Per cent efficacy of disease control.

used method against this disease. It is also having hazardous effect in our environment. Fungicides along with botanical combination approach provide a synergistic effect against the pathogen in the integrated disease management and are very effective. On the basis of results, the combination of Azoxystrobin 8.3% + Mancozeb 66.7% WG at 0.36% with Neem oil at 0.5% was observed to be superior over other treatments in management of Alternaria leaf spot of soybean.

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