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Effects of fertilizers and soil amendments on the incidence of Sclerotinia stem rot in Indian mustard (*Brassica juncea* L.)

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Indian mustard [*Brassica juncea* (L.)] is a major oilseed crop, grown in rabi season in India. Sclerotinia stem rot, stem blight or white rot disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a serious problem in mustard crop in Northern India. The present investigations were undertaken to understand the role of fertilizers and organic amendments on disease development in mustard crop under field conditions. In experiment 1, the effect of nitrogen, phosphorus and potash fertilizers with different levels on the development of Sclerotinia stem rot and on seed yield was studied during two years. The treatment was a combination of 80 kg N, 60 kg P and 60 kg K ha-^{1} which was applied significantly superior over control (without fertilizers) in disease control (60.42%) and increased seed yield (13.80 q ha-^{1}). Application of 100 kg N, and 60 kg P ha-^{1} without K was found to be least effective for disease management. In experiment 2, effect of five soil amendments, that is, mustard cake, neem cake, vermicompost, farm yard manure and sulphur on the development of Sclerotinia stem rot was studied. Neem cake applied at 1 ton ha-^{1} was found significantly superior with minimum disease incidence (20.43%), minimum disease intensity (15.00%), maximum disease control (63.27%) and increased seed yield (14.07 q ha-^{1}) over control (without amendments). The vermicompost and FYM applied at 15 ton ha-^{1} were also found effective in minimizing disease intensity and increased seed yield.

Key words: Fertilizers, Indian mustard, soil amendments and Sclerotinia stem rot.

INTRODUCTION

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is a major oilseed crop grown in rabi season in various regions of India. Rapeseed and mustard are the major oilseed crops of India with oil contents ranging between

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30 and 46%. Among the annual edible oilseeds, rapeseed and mustard contributes about 23% in acreage and over 25% in production for the last five years in India. India holds a leading position in area and production of rapeseed and mustard, that is,5.92 million hectares and 6.78 million tonnes, respectively, with an average productivity of 1145 kg ha^{-1}. The main mustard producing states in India are Rajasthan, Uttar Pradesh, Punjab, Haryana, etc. Among these states, Rajasthan stands first both in area and production, that is, 2.50 million ha and 2.97 million tonnes, respectively, with an average productivity of 1187 kg ha^{-1} (Directorate of Economics and Statistics, 2012). Rapeseed and mustard seeds are commonly used for the extraction of edible oil in Northern India. The oil content of the seeds ranges from 30 to 46%. The seed is used as a condiment in the preparation of pickles and for flavouring curries and vegetables. The oil cake is mostly used as a cattle feed. The seeds, oil and oil cakes are needed not only to meet the demand for internal consumption but also for export in order to earn the much needed foreign exchange.

Sclerotinia stem rot, or stem blight or white rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary has become a serious problem in mustard crop in northern India. *S. sclerotiorum* is soil-borne and has a large host range inciting more than 400 plant species (Boland and Hall, 1994). Sclerotinia stem rot is an economically important yield reducing disease that has been widely reported in the last few years in India and elsewhere. The high disease incidence and severe yield losses discourage farmers to grow the crop (Krishnia et al., 2000). The disease symptoms usually appear four to six weeks after sowing or at flowering stage, when significant damage has already been done. Sudden drooping of leaves followed by drying of plants are characteristic features of the disease. This disease is gaining importance in the mustard growing areas, which may cause crop failure as the disease incidence was recorded up to 73.8% in some districts of Punjab and Haryana (Kang and Chahal, 2000; Sharma et al., 2001). In Rajasthan, the disease incidence was recorded up to 60% (Ghasolia et al., 2004; Shukla, 2005). Various soil factors, such as nutritional status of the soil play a major role in the disease incidence. Sclerotia are the primary survival structures of *S. sclerotiorum* and act as a source of infection for many years in the field. Balanced fertilization and amendments of soil with decomposable organic matter could be an effective method of changing the soil and rhizosphere environment. Composts enrich the soil with microflora potentially competitive or antagonistic to pathogens or release inhibitory substances or volatiles during decomposition. The control of Sclerotinia stem rot of mustard in India is mainly achieved by using fungicides and cultural methods such as crop rotation and tillage to reduce inoculums of the pathogen. Most of the conventional methods are not effective in the management of S. sclerotiorum thus, the present study was undertaken to find out role of fertilizer doses (NPK) and soil amendments (neem cake, mustard cake, vermicompost, farm yard manure and sulphur dust) on disease severity and yield of mustard.

MATERIALS AND METHODS

Inoculum

An isolate of *S. sclerotiorum* was obtained from diseased mustard plant collected from farmer's field. Small pieces of diseased tissues along with adjoining healthy area and sclerotia found in diseased stem were surface sterilized by dipping in mercuric chloride solution (1:1000) for two minutes followed by three washings with sterile water and blot dried then plated aseptically on Potato Dextrose Agar (PDA) in Petri dishes. These were incubated in BOD incubator for growth of the fungus at 27 ± 2°C.

Sub cultures from pure peripheral growth were made on PDA slants. The pathogenicity of the isolated fungus was proved by mixing 15 day old inoculum (grown on sterilized sorghum grains) at the rate of 50 g per pot in the upper 3-5 cm layer of the sterilized soil of each pot. The soil of the pots were moistened and covered with polythene bags and left for 24 h in green house. On next day, apparently healthy surface sterilized mustard seeds were sown in these pots. Re-isolations from the diseased seedlings yielded the same fungus.

Mass multiplication of inoculum

The fungus inoculum was multiplied on sterilized sorghum grains. The sorghum grains were soaked in sterilized water overnight. The excess water drained out. Forty grams of grains was taken in each 250 ml conical flask, plugged with cotton and sterilized in an autoclave at 1.045 kg/cm^2 pressure for 20 min. The sorghum grains in flasks were inoculated aseptically with 5 days old mycelial discs (5 mm) of the pathogen and incubated for 15 days at 20 ± 2°C. The inoculum was mixed in rows at the time of sowing.

Two experiments were executed during rabi season of 2007-08 and 2008-09 at the research farm of the College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner. A randomized block design was used with three replications in 4 × 3 m^2 plots. The susceptible mustard cultivar 'varuna' was used for all experiments. All experiments were artificially inoculated with 20 g inoculums per meter row. All recommended agronomic practices were followed to raise the crop except fertilizers dose and organic amendments. The fertilizers and organic amendments were applied according to the treatments. Sowing of the crop was done during last week of October.

In experiment 1 the relationship of different fertilizers (NPK) and their combination with the disease development of Sclerotinia stem rot on mustard under field conditions were tested. The different treatments on combination of fertilizers N:P:K (kg ha^{-1}) viz. T_1-60:30:30, T_2-60:30:60, T_3-60:60:30, T_4-60:60:60, T_5-80:30:30, T_6-80:30:60, T_7-80:60:30, T_8-80:60:60, T_9-100:30:30, T_{10}-100:30:60, T_{11}-100:60:30, T_{12}-100:60:60, T_{13}-100:60:0 and T_{14}-80:30:0 were applied with control plot (T_{15}-without fertilizers). Half quantity of nitrogen, full quantity of phosphorus and potash were applied at the sowing time and remaining half quantity of nitrogen were applied in two splits at first and second irrigation.

In experiment 2, the effect of different soil amendments and their doses on disease development of Sclerotinia stem rot was studied. T_1&T_2-mustard cake 1 & 2 tons ha^{-1}, T_3&T_4-neem cake 0.5 & 1 ton ha^{-1}, T_5&T_6-vermicompost 10 & 15 tons ha^{-1}, T_7&T_8-FYM 10 & 15 tons ha^{-1} and T_9&T_{10}-sulphur 30 & 60 kg ha^{-1} were compared against a control plot (T_{11}-without organic amendments). All the amendments were applied in the plots before sowing of the mustard crop.

The observations of disease intensity, incidence and seed yield were recorded on plot basis. Disease intensity and incidence were recorded after 75 to 90 days after sowing. The results of experiments were statistically analyzed by using appropriate designs. To assess the Sclerotinia rot intensity, the rating (0-4) scale (Lesovoi et al., 1987; Sansford, 1995) was followed. 0 for healthy (No visible lesion), 1 for 0.1-2.0 cm lesion length on the stem, 2 for 2.1 - 4.0 cm lesion length on the stem, 3 for 4.1 - 6.0 cm lesion length on the stem and 4 for more than 6 cm lesion length on the stem or complete griddle plant. The length of lesion on infected stem was considered for recording the disease intensity (Sharma, 1987). The infected area was calculated from five randomly selected plants in each plot and then the average for each treatment was worked out. Percent disease intensity was calculated by using the formula [(sum of individual ratings/no. of plants observed × maximum disease rating) × 100]. The percent disease control was calculated by using the formula [(Disease in control - Disease in treatment/ Disease in Control) × 100] (McKinney, 1923).

RESULTS AND DISCUSSION

Experiment 1 revealed that the application of 80 kg N, 60 kg P and 60 kg K ha-^{1} was significantly superior in percent disease control (p=0.05) as well as in seed yield (13.80 q ha-^{1}) (p=0.05) over control (Table 1). Application of 100 kg N, and 60 kg P ha-^{1} without K was least effective for disease control (4.17%). Application of 80 kg N, 30 kg P without potash resulted in the lowest seed yield (6.59 q ha-^{1}). Table 1 suggests that application of more than 80 kg N ha-1 increase disease severity and reduced the percent disease control. This observation is in accordance with earlier findings of Gupta et al. (2004) that reported that increased nitrogen levels of nitrogen (60 to 90kg ha-^{1}) in soil led to significant increase in Sclerotinia stem rot disease incidence in mustard. In addition, Rathore and Chandawat (2003) found that increased nitrogen fertilization reduces seed yield and stover yield and increase susceptibility of blond psyllum to *Peronospora alta*, causing downy mildew. Chattopadhyay et al. (2002) observed that soil application of K fertilizer in *S. sclerotiorum* infected fields reduced the disease incidence in mustard. Banyal et al. (2008) reported nitrogen fertilization enhanced the development of collar rot caused by Sclerotium rolfsii in tomato, whereas phosphorus and potassium fertilization decreased the disease. Potassium (K) is essential for the synthesis of proteins, starch and cellulose in plants. Cellulose is a primary component of cell walls, and K deficiency causes cell walls to become leaky, resulting in high sugar (starch precursor) and amino acid (protein building blocks) concentrations in the plant. Nitrogen (N) is a key component of amino acids; therefore, an excessive supply of N can bring about higher amounts of amino acids and other N-containing compounds in plant tissues. These mineral imbalances lower resistance to fungal diseases by creating a more favorable environment for pathogens (Span and Schumann, 2010).

Experiment 2 revealed that the addition of neem cake, vermicompost, FYM, organic sulphur and mustard cake significantly decreased the disease intensity (p=0.05) in mustard as compared to the control. The addition of neem cake was significantly superior in disease control (p=0.05) as well as in increasing seed yield (Table 2). These findings are in agreement with those of Shivpuri et al. (1997) and Kapoor et al. (2006) who reported that neem based pesticides were highly effective against *Fusarium oxysporium* f.sp. *pisi*, F. *solani* f. sp. *pisi* and *S. sclerotiorum*. Neem cake possibly enhances the disease control by release of volatile substances during decomposition that induced disease resistance or tolerance on the root surface (Singh, 1983). It is a well-known fact that cakes improve physio-chemical properties of the soil and increase the vigour of the crop by supplying nutrients and promoting anti-microbial populations in the rhizosphere (Sharma and Sharma, 1986). Handoro et al. (2001) reported that application of soil amendments Neemax, FYM and sheep manure to pea crops decreased the disease incidence to S. sclerotiorum and increased seed yield. According to Tripathi et al. (2010), neem cake extract, mustard cake extract and farm yard manure extract reduces the mycelial growth of *S. sclerotiorum*. Sharma et al. (2011) found that the application of mustard cake reduced the stem rot incidence and increases seed yield in cauliflower. Generally, the integration of organic amendments and biocontrol agents have been found to improve the plant health in various crops (Rana et al., 2010; Chawla and Gangopadhyay, 2010; Yadav et al., 2013).

Thus, it was concluded that use of N fertilizer more than 80 kg ha-*1* increases Sclerotinia stem rot while proper application of K fertilizer and addition of organic amendments slowed down the Sclerotinia stem rot in mustard. Hence, balanced fertilizers and organic amendments are necessary for successful management of white stem rot of mustard and improve physio-chemical properties of the soil, increase the vigour of the plant by supplying certain nutrients and encourage anti-microbial population in the rhizosphere apart from providing reasonable disease control in the field experiments.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Table 1. Effect of fertilizer levels on disease development of Sclerotinia stem rot of Indian mustard under field conditions.

Treatment ertilizer dose (N:P:K) Kg ha^{-1}	Disease incidence (%)			Disease intensity (%)			Disease control (%)	Seed yield (q ha^{-1})			Increase in seed yield over check (%)
	2007-08	2008-09	Pooled	2007-08	2008-09	Average		2007-08	2008-09	Average	
T_1 60:30:30	36.67 (37.25)*	30.58 (33.35)	33.62 (35.39)	28.33 (32.13)	23.33 (28.84)	25.83 (30.49)	35.42	7.23	7.78	7.51	23.92
T_2 60:30:60	30.47 (33.48)	26.09 (30.70)	28.28 (32.09)	26.67 (31.06)	18.33 (25.30)	22.50 (28.18)	43.75	7.34	8.10	7.72	27.48
T_3 60:60:30	29.93 (33.14)	28.16 (32.03)	29.04 (32.59)	26.67 (31.06)	21.67 (27.70)	24.17 (29.38)	39.58	7.77	8.26	8.02	32.34
T_4 60:60:60	28.06 (31.95)	31.57 (34.17)	29.81 (33.06)	23.33 (28.84)	25.00 (28.84)	24.17 (29.38)	39.58	8.86	8.31	8.59	41.76
T_5 80:30:30	30.91 (33.74)	30.03 (33.21)	30.47 (33.47)	25.00 (29.99)	23.33 (28.84)	24.17 (29.42)	39.58	9.25	8.55	8.90	46.92
T_6 80:30:60	26.02 (30.60)	23.63 (29.06)	24.83 (29.83)	20.00 (26.55)	15.00 (26.55)	17.50 (24.67)	56.25	10.29	10.67	10.48	73.02
T_7 80:60:30	22.31 (28.16)	25.71 (30.44)	24.01 (29.30)	16.67 (27.04)	16.67 (22.59)	16.67 (32.66)	58.33	11.03	11.34	11.18	84.65
T_8 80:60:60	24.42 (29.59)	21.94 (27.91)	23.18 (28.75)	18.33 (25.30)	13.33 (14.75)	15.83 (23.31)	60.42	13.66	13.94	13.80	127.85
T_9 100:30:30	34.48 (35.94)	33.36 (35.27)	33.92 (35.61)	30.00 (33.15)	28.33 (32.13)	29.17 (32.64)	27.08	8.67	8.99	8.83	45.80
T_{10} 100:30:60	36.08 (36.90)	37.13 (37.53)	36.61 (37.21)	31.67 (32.13)	31.67 (35.24)	31.67 (34.22)	20.83	9.01	9.22	9.11	50.45
T_{11} 100:60:30	32.50 (34.68)	38.78 (38.47)	35.64 (36.57)	33.33 (34.22)	33.33 (34.22)	33.33 (35.24)	16.67	9.32	9.38	9.35	54.35
T_{12} 100:60:60	39.01 (38.63)	36.34 (37.06)	37.68 (37.84)	31.67 (32.13)	30.00 (33.20)	30.83 (33.71)	22.92	9.61	9.47	9.54	57.47
T_{13} 100:60:00	52.78 (46.58)	37.80 (37.91)	45.29 (42.24)	41.67 (40.18)	35.00 (36.26)	38.33 (38.22)	4.17	7.61	7.17	7.39	21.95
T_{14} 80:30:00	44.15 (41.62)	41.53 (4011)	42.84 (40.86)	36.67 (37.24)	33.33 (35.24)	35.00 (36.24)	12.50	6.59	6.60	6.59	8.88
T_{15} Control**	64.39 (53.35)	66.92 (54.58)	65.66 (54.11)	43.33 (41.15)	36.67 (37.24)	40.00 (39.20)	-	6.09	6.02	6.06	-
SEm ±	1.02	0.79	0.65	1.27	1.46	0.75	-	0.32	0.39	0.26	-
CD (P=0.05)	2.97	2.28	1.83	2.84	3.27	2.12	-	0.94	1.14	0.72	-

*Figures in parentheses are angular transformed values, **without fertilizers.

Table 2. Effect of soil amendments on disease development of Sclerotinia stem rot of Indian mustard under field conditions.

Treatment	Dose	Disease incidence (%)			Disease intensity (%)			Disease control (%)	Seed yield (q ha^{-1})			Increase in seed yield over check (%)
		2007-08	2008-09	Pooled	2007-08	2008-09	Pooled		2007-08	2008-09	Pooled	
T_1 Mustard cake	1 ton ha^{-1}	34.97 (36.24)*	38.63 (38.41)*	36.80 (37.32)*	30.00 (23.20)	31.67 (34.22)	30.83 (33.71)	24.49	8.19	7.69	7.94	12.98
T_2 Mustard cake	2 tons ha^{-1}	29.58 (32.93)	32.12 (34.48)	30.85 (33.71)	26.67 (31.06)	30.00 (33.20)	28.33 (32.13)	30.61	8.64	8.42	8.53	21.32
T_3 Neem cake	0.5 ton ha^{-1}	25.28 (30.17)	23.49 (28.98)	24.39 (29.57)	20.00 (26.55)	20.00 (26.55)	20.00 (26.55)	51.02	12.43	12.03	12.23	73.98
T_4 Neem cake	1 ton ha^{-1}	21.97 (27.90)	18.70 (25.54)	20.34 (26.72)	15.00 (22.78)	15.00 (22.78)	15.00 (22.78)	63.27	14.19	13.95	14.07	100.18
T_5 Vermicompost	10 tons ha^{-1}	35.92 (36.80)	37.33 (37.63)	36.63 (37.22)	23.33 (28.84)	33.33 (35.24)	28.33 (32.04)	30.61	11.08	10.52	10.80	53.67
T_6 Vermicompost	15 tons ha^{-1}	24.48 (29.63)	32.20 (34.49)	28.34 (32.06)	18.33 (25.30)	28.33 (32.13)	23.33 (28.71)	42.86	12.15	11.90	12.03	71.10
T_7 FYM	10 tons ha^{-1}	41.94 (40.34)	44.18 (41.61)	43.06 (40.99)	33.33 (35.24)	33.33 (35.24)	34.17 (35.75)	16.33	10.90	9.19	10.05	42.94
T_8 FYM	15 tons ha^{-1}	36.19 (36.96)	37.19 (37.56)	36.69 (37.26)	31.67 (34.22)	31.67 (34.22)	31.67 (34.22)	22.45	11.94	11.76	11.85	68.65
T_9 Sulphur	30 kg ha^{-1}	30.99 (33.80)	30.18 (33.29)	30.58 (35.55)	21.67 (27.70)	25.00 (29.99)	23.33 (28.84)	42.86	8.75	8.81	8.78	24.90
T_{10} Sulphur	60 kg ha^{-1}	26.30 (30.81)	23.30 (28.84)	24.80 (39.82)	20.00 (26.55)	21.67 (27.70)	20.83 (27.13)	48.98	9.05	10.19	9.62	36.83
T_{11} Control**		60.34 (50.95)	61.91 (51.87)	61.13 (51.41)	38.33 (38.23)	43.33 (41.15)	40.83 (39.69)	-	6.99	7.07	7.03	-
SEm ±		0.96	1.22	0.78	1.40	1.21	0.60	-	0.40	0.40	0.29	-
CD (P=0.05)		2.84	3.59	2.24	2.68	2.31	1.72	-	1.18	1.18	0.82	-

*Figures in parentheses are angular transformed values, **without soil amendments.

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