Effect of Neem Powder on Growth of *Macrophomina phaseolina* and Charcoal Rot Incidence in Common Bean

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ABSTRACT

Powder of neem seed and leaves (NSP and NLP) significantly reduced the myceial growth of the fungus. Maximum reduction was brought about by 4 or 5g of NSP. The average diameter of the colony recorded in case of two weights was nearly 3.3 cm compared to 7.2 cm in the control plates. In non- sterilized soil, NLP reduced the disease incidence by 44.2% and 38% in season, 2001/02 and 2002/03 respectively as compared to the control. However, the reductions in disease incidence were significantly lower in sterilized soil, they respectively 18.6% in 2002/03 and 22% in seasons 2003/04. NLP was followed by the neem seed kernel powder (NSKP) in its effect on disease incidence. Benomyl at 2g/kg seeds was the most effective treatment. Reduction brought about the fungicide amounted to 83.4% and 45.8%in non- sterilized and sterilized soils respectively. Disease incidence increased with the added amount of the powder from each of the parts used in case of the non- sterilized soil, but the reverse was obtained when the soil was sterilized.

INTRODUCTION

The agricultural sector plays the major role in the economy of the Sudan and the production of legume crops in widely distributed throughout the country. Common bean (*Phaseolus vulgaris*) is grown in the Sudan in vast areas in both River Nile and Khartoum States and in considered the second most important food legume next to *Faba bean*. It is also second to cereals in their importance as a human food. In Sudan common bean is subject to several serious diseases and the charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid is by the most important fungal disease that limits production of crop in River Nile State. The use of synthetic chemical compounds for production of agriculture crops may showed side – effects. This has neceritated the search for safe chemicals particularly of plant origin (botanicals) for the purpose of crop protection. The objective of this work is to investigate the effectiveness of powdery obtained from different Neem parts against charcoal rot disease of common bean

MATERIAL AND METHODS

laboratory trail

The effect of Neem Seed and Leaf Powder on the Linear Growth of the Pathogen

Six weights were used for each powder, i.e. 0.5, 1.0, 2.0, 3.o, 4.0, and 5.0g. Each of the weights was added to 100 ml PDA medium in a 250 ml Erlenmeyer flask and then volume of 15ml was poured into each of 3 – Petri dishes. Amyceial disk (5mm in dia) was taken from the periphery of an actively growing fungal colony and placed on the surface of the medium in each plate. Growth rate per 12 hr was determined.

Preparation of the Fungus Inoculum and Inoculation Method

The fungus was cultured on PDA for 3- days at room temperature. Five myceial disks (5 – mm in dia.) were taken from the margin of an actively growing colony and then placed on the surface of sterilized rice seeds (200g) in a flask into which distilled water (200ml) was added and autoclaved for 20 min. after inoculation, all flasks were placed on a bench in the inoculation room for 15 days at room temperature (songa, et. al. 1997). Six rice seeds completely covered with selerotia of the fungus were placed with the seeds of common bean in each hole at sowing and then covered with soil.

The Effect of Powders Prepared from Different Neem Parts on Charcoal Rot on Common Bean.

Neem leaves (NL), kernels (k) and whole seeds (s) were collected, air – dried and crushed using pestle and mortar. The powder from each part was then mixed with the soil in clay pots containing 3 kg soil each at rates of 10, 20, 30g/kg soil. Treatments were replicated thrice in an RCB design. Seeds of the two common bean varieties, R0/2/1 and Giza -3 were sown at the rate of 5-seeds per post. Artificial inoculation with fungus was carried out and as controls bean seeds were either treated with Benomyl at the rate of 2g/kg seed or untreated and sown in untreated (un inoculated) soil. Data on dead and parently healthy plants were collected over a period of one and half month after emergence. The trail was conducted during the two seasons 2001/02 and 2002/03 in sterilized and non – sterilized soils.

RESULTS

The effect of Neem seed powder (NSP) on the radial growth of the pathogen:

Results summarized in Table 1 show that NSP tested at 1.0, 2.0 3.0 4.0, and 5.0g per 100 ml PDA reduced the radial growth of *Macrophomina*

Phaseolina, and differed significantly from the control in the diamter of the fungal colony. However, when the powder was tested at 0.5g there was no significant difference from the control. As the amount of the powder increased, the rate of the fungal growth decreased. Reduction in the growth rate contined with time. The lowest growth rates in almost all treatments were obtained at 48 hr, except for the control and when powder was tested at 0.5g. In both cases, it was not possible to measure the rate of growth after 36hr. as the fungus completely covered the petri dish after a few hours after the first day.

Table 1: Average radial growth of the mycelia (cm) of *M. phaseolina* on PDA treated by Neem Seed Powder (NSP)

NSP/100 ml PDA (g)	Radial growth (cm) at			
	12 hr	24 hr	36 hr	48 hr
Control	4.317	8.117	9.0	9.0
0.5	4.467	8.917	9.0	9.0
1.0	2.167	2.850	4.08	4.380
2.0	2.150	3.150	3.917	4.190
3.0	1.650	3.350	3.450	3.850
4.0	1.083	2.383	2.933	3.125
5.0	1.150	2.567	3.217	3.417
SE ±	0.64***	0.91***	0.99***	1.09***
CV%	11.13	2.85	2.55	2.44

Effect of Neem leaf powder (NLP) on the radial growth of the pathogen.

As indicated in table 2 NLP differed significantly from the control in the diameter of the fungal colony, however, no significant difference was found after 24hr. when the powder was tested at 0.5g. The rate of the fungal growth decreased with the increase in the amount of the powder used.

Table 2: Average radial growth of mycelium (cm) of *M. phaseolina* on PDA treated by Neem Leaf Powder (NLP)

NLP/100ml PDA (g)	Radial growth (cm) at			
	12 hr	24 hr	36 hr	48 hr
Control	3.333	3.500	8.867	9.000
0.5	3.167	3.333	8.067	8.233
1.0	2.933	3.067	8.033	8.333
2.0	2.800	2.900	8.033	8.233
3.0	2.733	2.867	7.767	8.000
4.0	2.700	2.733	7.533	7.667
5.0	2.467	2.500	7.500	7.600
SE ±	0.23*	0.28*	0.27**	0.28**
C.V.%	8.26	1.76	1.95	2.01

Effect of Powders from Different Neem Parts on Incidence of Charcoal Rot in non – Sterilized Soil.

Results obtained are presented in table 3- A and B for seasons 2001 / 02 and 2002 /03 respectively. Powder of the whole Neem seeds (NS), kernel (NSK) and leaves (NL) significantly ($p = 0.05$) reduced the disease incidence compared to the untreated control. The fungicide Benomyl was the most effective treatment as it consistently showed the lowest disease incidence throughout the growth period of common bean powders from NL and NSK showed similar effects on charcoal rot incidence in care of R0/2/1 variety. Both were better than NS powder. However, results obtained with the other variety (Giza - 3) showed that NL powder was the best as evidenced by the significantly lower charcoal rot incidence compared to the other treatment. Not withstand the fact that powders from the different neem parts substantially reduced the disease incidence as compared to the control, charcoal rot incidence increased with the increase in the powder concentration. Table 4 (A and B). Results obtained with either of two common bean varites showed more or less the same trends in the two seasons. Ro/2/1 showed consistently higher incidence in the two seasons (table 5 A and B). The treatment combinations showed non significant effect on disease in some cases (table 6 (A and B)).

Table 3: Effect of neem powder from different neem plant parts on charcoal rot incidence in non-sterilize soil

(A) Season 2001-02

Treatment	Weeks After Sowing (WAS)			
	2	3	4	5
Control	36.6	50.0	60.0	63.3
NSK	17.7	29.9	34.9	36.2
NS	31.3	38.2	43.0	44.4
NLP	10.4	20.5	27.0	29.1
Benomyl (2g/kg seed)	0.57	6.6	13.3	13.3
SE ±	2.71***	2.05***	1.46***	1.6***
C.V %	58.5	29.5	17.8	18.5

(B) Season 2002 - 03

Treatment	Weeks After Sowing (WAS)			
	2	3	4	5
Control	60.0	60.6	63.3	96.7
NSK	23.4	38.3	43.7	65.1
NS	42.4	46.4	53.3	73.3
NLP	26.8	28.3	34.5	58.2
Benomyl (2g/kg seed)	0.57	6.6	13.3	13.3
SE ±	2.8***	2.36***	2.56***	3.24***
C.V %	38.5	26.6	24.8	21

NSK= Neem seed kernel, NS= Whole seed, NLP= Neem leaf powder
WAS= weeks after sowing

Table 4: Effect of different amounts of powder from different neem parts used in non- sterilized soil on charcoal rot incidence

(A) Season 2001- 02

Treatment	WAS			
	2	3	4	5
10(g)	11.1	19.8	26.4	26.4
20(g)	17.5	28.5	34.3	36.3
30(g)	30.7	40.3	44.3	47.0

Benomyl (2g/kg seed)	0.57	6.6	13.3	13.3
Control	36.6	50.0	60.0	63.3
SE ±	2.71***	2.05***	1.46***	1.60***
C.V %	58.5	29.5	17.8	18.5

(B) Season 2002/03

Treatment	WAS			
	2	3	4	5
10 (g)	24.7	30.5	31.1	56.8
20 (g)	32.6	41.0	48.5	63.7
30 (g)	35.3	41.5	51.8	76.0
Benomyl (2g/kg seed)	0.57	6.6	13.3	13.3
Control	60.0	60.6	63.3	96.7
SE ±	2.80***	2.36***	2.56***	3.24***
C.V %	38.5	26.6	24.8	21.0

WAS= Weeks after sowing

Table 5: Response of two common bean varieties to different neem parts concentration in non- sterilized soil in (A season 2001/02 and B season
(A)

Treatment	**WAS**			
	2	**3**	**4**	**5**
R0/2/1	19.5	32.8	37.0	38.8
Giza -3	20.1	26.3	32.9	34.3
SE ±	2.21**	1.68**	1.20**	1.30**
C.V.%	58.1	29.5	17.8	18.5

(B)

Treatment	**WAS**			
	2	**3**	**4**	**5**
R0/2/1	36.9	42.4	47.0	72.5
Giza -3	24.8	32.9	40.6	58.5
SE ±	2.29***	1.93***	2.09***	2.64***
C.V.%	38.5	26.6	24.8	21.0

WAS= Weeks after sowing

Table 6: Effect of different amounts (in g) of powders from different neem parts on charcoal rot incidence in season 2001/02 (A) and 2002/03 (B)

(A) Season 2001/02

2 weeks after Sowing

Parts / Concentrations	NSP	NSK	NLP
10	24.3	8.9	0.1
20	30.4	17.7	4.5
30	39.2	26.4	26.6

SE± = 4.7 C.V.= 58.1%

3 weeks after sowing

Parts / Concentrations	NSP	NSK	NLP
10	30.8	19.8	8.9
20	36.9	30.8	17.7
30	46.9	39.0	35.0

SE ± =3.6 C.V.%= 29.5%

(A) Season 2001/02

4 weeks after sowing

Parts / Concentrations	NSP	NSK	NLP
10	37.1	28.7	13.3
20	41.2	32.9	28.7
30	50.8	43.1	39.0

SE ± =2.3 C.V.%= 17.7%

5 weeks after sowing

Parts / Concentrations	NSP	NSK	NLP
10	37.1	28.7	13.3
20	43.1	35.0	30.7
30	52.9	45.0	39.0

SE ± =2.8 C.V.% = 18.5%

(B) Season 2002/03
2 weeks after sowing

Parts / Concentrations	NSP	NSK	NLP
10	41.1	24.1	8.9
20	41.1	19.8	36.9
30	45.0	26.4	34.6

SE ± =4.9 C.V. %= 38.5%

3 weeks after sowing

Parts / Concentrations	NSP	NSK	NLP
10	41.1	36.9	13.3
20	49.2	36.9	36.9
30	49.0	41.1	34.6

SE ± = 4.1 C.V. %= 26.6%

(B) Season 2002/03
4 weeks after sowing

Parts / Concentrations	NSP	NSK	NLP
10	43.0	36.9	13.3
20	59.0	45.0	41.1
30	57.1	49.0	49.2

SE ± = 4.4 C.V. %= 24.8%

5 weeks after sowing

Parts / Concentrations	NSP	NSK	NLP
10	66.0	59.4	45.0
20	68.1	63.7	59.4
30	85.6	72.3	70.2

SE ± = 5.6 C.V.%= 21%

Effect of Powders of Different Neem Parts on Incidence of Charcoal Rot in Sterilized Soil

Results obtained in seasons 2002/03 and 2003/04 indicated respectively in table 7A and 7B. Generally, NS/ NSK and NL powders significantly (p= 0.05) reduced charcoal rot incidence, compared to the untreated control. Again the fungicide Benomyl (2g/kg seed) was the most effective in reducing disease incidences and it gave consistent results NSK powder showed similar effect to powders obtained from NL or NS as recorded in the some cases showed similar effects.

In general NL powder was the most effective among all others in causing significant reduction in disease incidence. Contra sty to the previous trial disease incidence decreases with the increase in the amount of powder applied to the soil (table 8 (A and B)). Results obtained in the two seasons were comparable.

Table 7: Effect of different neem parts powder on charcoal rot incidence in sterilized soil

(A) Season 2002-03

Treatment	WAS			
	2	3	4	5
NSK	12.4	15.9	24.6	33.2
NSP	22.0	23.7	31.3	39.7
NLP	10.7	14.1	21.1	28.0
Benomyl (2g/kg seed)	0.57	0.57	6.6	20.0
Control	26.56	35.0	39.23	46.6
SE±	2.50	2.43	2.64	1.54
C.V %	64.39	52.64	39.82	18.46

(B) Season 2003-04

Treatment	WAS			
	2	3	4	5
NSK	15.24	23.01	32.12	37.08
NSP	22.77	27.08	36.19	42.85
NLP	18.74	19.59	27.08	33.01
Benomyl(2g/kg seed)	0.1	0.1	0.1	9.23

Control	30.78	35.01	51.15	54.99
SE±	2.93	2.71	2.90	2.28
C.V %	41.71	37.71	26.74	27.26

WAS= Weeks after sowing

Table 8
Effect of different amounts of neem powder on charcoal rot incidence in sterilized soil
(A) Season 2002- 03

Treatment	WAS			
	2	3	4	5
10(g0	24.9	27.8	37.4	44.5
20(g)	14.8	17.7	25.0	33.5
30(g)	8.9	8.9	17.7	20.7
Benomyl(2g/kg seed)	0.1	0.1	8.9	26.6
Control	26.7	35	39.2	43.1
SE±	2.50	2.43	2.64	1.94
C.V %	64.4	52.6	39.8	18.4

(B) 2003- 04

Treatment	WAS			
	2	3	4	5
10(g)	34.34	34.76	45.77	51.41
20(g)	23.6	29.38	33.6	39.1
30(g)	6.34	16.42	27.9	33.47
Benomyl(2g/kg seed)	0.1	0.1	0.1	9.23
Control	30.78	35.01	51.15	54.99
SE±	2.93	2.71	2.90	2.28
C.V %	41.7	37.71	26.74	27.26

DISCUSSION

The use of neem derived compounds, including seed kernel leaves and ground seeds proved effective against *M. Phaseolina* both in pot and in laboratory tests. However, when non – sterilized soil was amended with a relatively high amount of ground neem seed (30g/kg soil), disease incidence increased significantly as contrasted to the sterilized soil. It seems likely that the antagonistic micro organism in the natural soil were more sensitive than the pathogen to the fungi toxic active ingredients released from powdered neem seeds, and the inhibitory effect was concentration dependent. In the soil amended with small amount of the powdered seeds (10/kg soil), the inhibitory effect might be overcome by the stimulatory effect brought about by nutrients supplied by the powdered seeds, this could subsequently result in suppression of the pathogen through the antagonistic effect of the micro organisms in the soil and/ or competition with the pathogen. However, this explanation is only inferential but it needs to be experimentally verified. Results clearly indicated that leaves and seed kernels were more effective in reducing disease incidence than the whole seed. It is a well established fact that neem leaves contain anti fungal compounds. As reported by Jeyarajan et al. (1986) incorporation into the soil of dried neem leaves resulted in significant reduction in wilt incidence of betevine (*Piper betle*) caused by *Phytophthora capsici*. Adeline in the population of *Pythium aphanidermatum* was achieved by incorporation of green neem leaves into infested soil (Singh and Parly, 1996). Similarly, the total fungal population in tomato rhizosophere was reduced by application of chopped neem leaf amendment (prakash, 1985). Aqueous leaf extract has also been reported to inhibit a number of soils – borne pathogens of chikpea (*Cicer arietinum*). These include *Fusarium exysporum f. sp. Cicer*, *Rhizoctonia Solani*, *sclerotum rolfsii* and *sclerotinia sclerotiorum* (Singh, 1980). Inhibition of foliar diseases by aqueous leaf extracts has been repented by other investigators (Rhownick and Vandhan, 1981).

The Apparently more effect of seed kernels against the pathogen as compared to the whole seeds could be mainly due to the relatively high concentration of neem seed oil in the kernels, the oil is known to contain a number of biologically active compounds, and its application in agricultural systems for crop protection is being explored. In a study on the effect of neem seed oil and various aqueous neem extracts on four soils – borne pathogens, the oil was found most inhibitory, a result which might suggest the presence of anti fungal substances in the oil (Singh, 1980). This result may also explain the higher inhibitory effect shown by seed kernels as compared to other neem – drived compounds when used against *M.*

phaseolinna in this study. The inhibitory effect of the oil against several foliar pathogens, including powdery mildew and rust pathogens have been reported (Chiamella and Roveshi, 1992). l neem –According to Achimu and Schlosser, 1992 Powdery mildew of grape caused by *plasmopora viticola* is sensitive to several neem- derived extracts including neem oil. Both neem oil and leaf extracts were also found effective against two phytopathogenic fungi (*Heliminthosporium nodilosum* and *Pyricularia oryzae*) attacking leaves of finger millet (Jagannathan and Narasimhan, 1988).

Conclusions

Charcoal rot is an important biotic constraint to common been production in River Nile State Powders of neem seed kernel and leaves applied to culture medium displayed inhibitory effects to the growth of the fungus. Leaf powder proved the best as it caused up to 36.3% reduction in disease incidence in non – sterilized soil.

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