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Evaluation of varying concentrations of neem seed extracts in the control of cowpea scab (*Sphaceloma* SP.)

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Abstract

Cowpea scab caused by *Sphaceloma* sp. is the most destructive fungal disease of cowpea in northern guinea savannah ecological zone of Nigeria and indiscriminate use of synthetic fungicides in the control of the disease is posing serious threats to the environment and development of resistance strains. Field experiment was therefore, conducted in Bauchi-Nigeria to evaluate different concentrations of neem seed extracts as safe, cheap and environmentally friendly strategy in the management of cowpea scab. The treatments involved three neem seed formulations namely: Aqueous Neem Seed Extract (ANSE); Neem seed Oil (NSO); and Emulsifiable Neem Seed Oil (ENSO) each evaluated at three different concentrations (10, 15, 20% ANSE; 2, 3, 4l/ha NSO and 1:05, 1:10, 1:15v/v ENSO) along with standard fungicide mancozeb (2.5kg/ha) as check and unsprayed plots serving as control. The neem extracts were applied at weekly intervals and cowpea variety IT88D-867-11 which was highly susceptible to scab was used. Disease incidence and severity were assessed at weekly intervals. The results shows that neem seed extracts reduced the incidence by 8-26%and severity by 5-49%more than the unsprayed plants. Application of 1:05, 1:10v/v ENSO; 15, 20% ANSE was as effective as mancozeb in reducing severity of scab on cowpea stems, peduncles and pods. Cowpea yield loss reduction ranges from 19 – 75% on neem treated plants than the control. Application of 1:05, 1:10 ENSO and 15, 20% ANSE is therefore recommended in the management of cowpea scab infection and can be use as components of cowpea production package.

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Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important staple grain legume providing major source of dietary protein to an average man in West Africa. In Nigeria, almost all parts of the crop are utilized by the rural people for various dietary preparations. Besides its usefulness in human diets, the crop is also used as fodder for animals and in soil conservation practices (Bationo*et al.,* 2002). The crop is frequently grown in mixture with other crops like maize, sorghum and millet, but sometimes as sole crop (Mortimore *et al.,* 1997; Olufolaji and Singh, 2002).

The bulk of cowpea produced in Africa is grown under subsistence agriculture with an average yield of less than 1.0t/ha. In Nigeria, the annual production of cowpea was estimated to be about 2.95 million tones (FAOSTAT, 2015). This accords Nigeria the leading producer of the crop in the world.

In spite of the importance of cowpea to Nigerian economy, reduction in yields in the production areas is recorded largely due to a number of factors including poor soil, insect pests and diseases and drought (Bationo *et al.,* 2002). Cowpea diseases induced by various pathogenic groups (fungi, bacteria, viruses, nematodes and parasitic higher plants) constitute one of the major constraints to cowpea production in all agro-geopolitical zones where the crop is grown (Emechebe and Lagoke, 2002).

The most destructive fungal disease of cowpea in Northern Nigeria is cowpea scab caused by *Sphaceloma* sp. (Emechebe, 1980; FAO, 1993). The disease affects all the above ground parts of the plant including leaf, stem, peduncle, petiole and pod. Crop loss due to scab ranges from 60 to 100% in northern guinea savanna of Nigeria where the crop is largely grown (Mungo *et al.,* 1995). Thus an urgent attention is therefore required to reduce the losses caused by the disease so as to increase cowpea yield and improve farmer's income. The objective of this research therefore, was to evaluate the efficacy of different concentrations of neem seed extracts in the management of cowpea scab disease.

Materials and methods

The experiment was conducted in Abubakar Tafawa Balewa University Teaching and Research Farm, Bauchi-Nigeria. Bauchi is located on latitude 10°17'N and longitude 9°49'E situated at 690.2m above sea level. The climatic conditions are characterized by dry (November to March) and wet (April to October) seasons.

The experimental field was ploughed and harrowed to obtain a uniform soil gradient and fine tilth for root penetration. The field was marked out in to plot sizes of 2m x 4m with pegs. The plants were spaced 20cm x 50cm within and between rows, respectively. Plots were separated from one another by one-meter border rows where two rows of soybean were grown to reduce pesticide drift. The cowpea variety used for the experiment is IT88D-867-11, which was known from previous field observations to be highly susceptible to cowpea scab in Bauchi.

Preparation of neem seed extracts

Three different neem seed formulations namely Aqueous Neem Seed Extract (ANSE), Neem Seed Oil (NSO) and Emulsifiable Neem Seed Oil (ENSO) were prepared and used in the experiment. The preparation of each extract was described below.

Preparation of aqueous neem seed extract

Fresh matured healthy neem seeds which had fallen on ground were collected underneath the neem trees in Government Science Secondary School, Gombe-Nigeria. The seeds were dried under shade for seven days to reduce moisture in the shells for easy cracking. The shells were cracked using mortar and pestle to remove the kernels. Five hundred, 750 and 1000g of the neem kernels were weighed in the laboratory using Mettler PC2000 weighing balance. The kernels were separately pulverized using mechanical blender until they formed paste. The paste was then wrapped in a clean cloth and then suspended in four litres of water for 24hours. The soaked pulverized neem kernels was squeezed inside

the cloth using both hands before filtering using two folds muslin cheese cloth. Tap water was then added to make the volume of each of the filtrate to five litres. This gave 10, 15 and 20% concentrations of the neem seed extract. The filtrate was sprayed on scab infected cowpea plants using knapsack sprayer.

Extraction of neem seed oil

Neem kernels were crushed and ground in to powder as described above. The powder was sieved using sieve of 0.5mm mesh size. The larger kernel particles were further grounded and sieved. The kernel powder was then emptied into plastic bowl measuring 50cm and 30cm top and bottom diameters respectively. Water was added to wet the kernel powder at 25ml/kg v/w. The powder was mixed thoroughly with water using both hands until a hard mass was formed. The mass formed was placed under the sun for 30minutes and thereafter the mixing continued in the bowl with both hands until oil started appearing in the bowl. From the hard mass of the neem kernel, a handful was then cut and squeezed with a hand to press out the oil. This process continued until all the mass had been pressed and oil removed from it.

The oil collected was then filtered through a twofold muslin cloth to remove solid particles and stored in the dark. Different concentrations (2.0, 3.0, and 4.0l/ha) of the oil were applied using ultra low volume applicator (ULVA+ sprayer) by varying the spray volume through sprayer calibration.

Preparation of emulsifiable neem seed oil

One hundred and twenty five grams of caustic soda (sodium hydroxide) was weighed and dissolved in one litre of distilled water inside five-litre capacity plastic bucket. The solution formed was added to two litres of neem seed oil as described by stoll (1998) and Tijjani *et al.* (2014).

The solution formed was stirred vigorously with clean wooden stirrer to produce a mixture called mother extract (ME). Known volume of the ME was added to water in different rations (v/v) (1:05, 1:10, and 1:15 of ME:water) and applied on scab infected cowpea plants using ULVA+ sprayer.

Experimental design

The treatments consisted of three neem seed formulations each with three concentrations: 10, 15 and 20% for ANSE; 2.0, 3.0 and 4.0l/ha for NSO; and 1:05, 1:10 and 1:15v/v (ME:water) for ENSO, respectively. Included in the treatments were synthetic fungicide (mancozeb) at 2.5kg/ha and plots that were sprayed with water only serving as control. The treatments were combined in a nested design and laid in a randomized complete block design with three replications. Ten days after sowing the seedlings were inoculated by placing the previously preserved scab-infected cowpea residues near the plants' canopy as described by Mungo *et al.,* (1995). At three weeks after sowing spray application of the different concentrations of neem seed extracts started between 7.00am and 10.00am local time at weekly intervals. Manual weeding using hoe was carried out two times at four and eight weeks after sowing.

Disease assessment was made at weekly intervals beginning from three weeks after sowing. Ten plants in the two central rows in each plot were tagged for data collection. Disease incidence was recorded by counting number of plants with symptoms of the disease and dividing by the total number of plants assessed and the result converted to percentage as shown below:

$$\text{Disease incidence} = \frac{Number\ of\ diseased\ plants}{Number\ of\ plants\ assessed} \times 100\%$$

Disease severity was recorded weekly on leaf, stem, peduncle and pods using visual scale developed by Allen *et al.,* (1981) where 1 = no symptoms and 10 = severe damage on plant parts. Other data collected included number of pods per plant by simply counting the total number of pods in the effective rows and taking average, seeds per pod by randomly picking ten pods at random and counting the number of seeds and the average recorded for each plot. Other data included total pod yield (kg/ha) by taking the weight of the total pods, and seed yield by weighing the total seeds in each plot.

The data collected were subjected to analysis of variance using Mstat-C statistical package developed by Russell D. Freed, Michigan State University, USA. Treatment means were separated using Duncan's multiple range test.

Results

Disease incidence

Results on the incidence of cowpea scab as influenced by spraying with varying concentrations of neem seed formulations are presented in Table 1. Evidently, the incidence varied significantly ($P \leq 0.01$) with the treatments. The incidence of scab on plants sprayed with 1:05 v/v ENSO was significantly lower than on plants treated with 2.0l/ha NSO, 1:15v/v ENSO, 10% ANSE and the control. However, plants sprayed with 3.0 and 4.0l/ha NSO, 1:10v/vENSO, 15 and 20% ANSE were infected by *Sphaceloma* pathogen as those sprayed with 1:15 ENSO. Their effectiveness in reducing the incidence of the disease is comparable to the synthetic fungicide mancozeb. Although, scab occurrence on plants treated with 3.0l/ha NSO, 1:10 and 1:15v/v ENSO was lower, the prevalence of the disease however, did not differ significantly ($P \leq 0.01$) from those treated with 10% ANSE.

The scab incidence was higher on the control plants than those treated with neem seed formulations except with those sprayed with 2.0l/ha NSO.

Table 1. Effect of spraying varying concentrations of neem seed extracts on the incidence of scab on cowpea.

Neem Extracts	Dosage	% Incidence
NSO (l/ha)	2.0	75.2ab
	3.0	65.2c-e
	4.0	61.7de
Mean		67.4
ENSO (v/v)	1:05	60.2e
	1:10	66.4c-e
	1:15	68.8b-d
Mean		65.1
ANSE (%)	10	71.0bc
	15	60.7de
	20	60.7de
Mean		64.0
Mancozeb (kg/ha)	2.5	62.4de
Control	0	81.4a
Overall mean		66.7
SE±		2.02

NSO = neem seed oil; ENSO = emulsifiable neem seed oil; ANSE = aqueous neem seed extract

Means followed by the same letters within a column are statistically the same.

Disease severity

The severity of cowpea scab was assessed on four infected cowpea aerial parts namely leaf, stem, peduncle and pod and the results presented in Table 2. The severity of scab on cowpea infected plant parts varied significantly ($P \leq 0.01$) with the application of varying concentrations of different neem seed formulations. Plants sprayed with different neem seed formulations showed lower disease severity than the control. The severity on cowpea leaves was lower on plants sprayed with 1:05v/v and 1:10v/v ENSO than those sprayed with all dosages of NSO, ANSE, 1:15v/v

ENSO and the control. The superiority of 1:05v/v and 1:10v/v ENSO over other dosages in reducing leaf scab was comparable to that of mancozeb. Application of 4.0l/ha NSO, 1:05 or 1:10v/v ENSO and 15 or 20% ANSE reduced severity of scab on cowpea stem comparable to application of mancozeb. Scab severity on cowpea peduncle and pod was significantly ($P \leq 0.01$) reduced on plants sprayed with 1:05 or 1:10v/v ENSO and 15 or 20% ANSE comparable to plants sprayed with mancozeb. Infection of cowpea parts was more severe on plants sprayed with NSO than those sprayed with ANSE or ENSO.

Table 2. Effect of spraying varying concentrations of neem seed extracts on the severity of scab on infected cowpea plant parts.

Neem Extract	Dosage	Leaf	Stem	Peduncle	Pod
NSO (l/ha)	2.0	6.9a	6.3a	6.6b	6.8ab
	3.0	5.5bc	5.1bc	5.1cd	6.0bc
	4.0	5.5bc	4.8cd	4.8c-e	5.4cd
Mean		6.0	5.4	5.5	6.1
ENSO (v/v)	1:05	3.8f	4.2d	3.8f	3.8e
	1:10	4.2ef	4.7cd	4.0ef	4.4de
	1:15	5.0cd	5.5b	4.9cd	5.2cd
Mean		4.3	4.8	4.2	4.5
ANSE (%)	10	5.7b	5.6b	5.5c	5.4cd
	15	4.8de	4.8cd	4.3d-f	4.9c-e
	20	4.5de	4.5cd	3.8f	4.6de
Mean		5.0	5.0	4.5	5.0
Mancozeb (kg/ha)	2.5	3.8f	4.3d	3.9f	3.9e
Control	0	7.3	6.8a	7.5a	7.6a
Overall mean		5.2	5.2	5.0	5.3
SE±		0.16	0.16	0.21	0.29

NSO = neem seed oil; ENSO = emulsifiableneem seed oil; ANSE = aqueous neem seed extract

Means followed by the same letters within a column are statistically the same.

Yield components and grain yield

The results for the effect of controlling cowpea scab with varying concentrations of neem seed formulations on yield components and grain yield of cowpea are presented in Table 3. Number of pods per plant was influenced significantly ($P \leq 0.01$) by spraying different concentrations of neem seed formulation. Pods produced on plants sprayed with 3.0 or 4.0l/ha NSO; 1:05, 1:10 or 1:15v/v ENSO; and 15 or 20% ANSE were statistically the same, but differed from those produced on the control plants. Number of pods obtained on plants sprayed with 4.0l/ha NSO; 1:05, 1:10 or 1:15 ENSO; and 15 or 20% ANSE were comparable to that obtained on plants treated with mancozeb. All plants treated with the neem formulations produced more number of pods than the control plants. Spraying different concentrations of neem seed formulations resulted in significantly ($P \leq 0.01$) greater number of seeds per pod from among treated plants than from the control plants. Plants sprayed with 3.0 or 4.0l/ha NSO; 1:05, 1:10 or 1:15v/v ENSO; and 10, 15 or 20% ANSE produced significantly ($P<0.01$) more seeds/pod comparable to plants treated with mancozeb. Cowpea pod yield significantly ($P \leq 0.01$) differed with spraying varying concentrations of neem seed formulations. Noticeably, the pod yield obtained from treatment of cowpea with 1:05v/v ENSO (2,702kg/ha) was

comparable with that obtained from cowpea plants treated with 1:10v/v ENSO (2,521kg/ha), 20% ANSE (2,463kg/ha) and mancozeb (2,716kg/ha) respectively. The lowest pod yield (1,194kg/ha) was recorded on the control plants, which was lower than the treated plants. Similarly, grain yield differed significantly ($P \leq 0.01$) with the application of varying concentrations of neem seed formulations. Cowpea grain yield was significantly higher on those plants treated with 1:05v/v ENSO (1,969kg/ha) than those plants sprayed with 2.0 (941kg/ha), 3.0 (1,315kg/ha) or 4.0l/ha (1,469kg/ha) NSO; 1:15v/v (1,505kg/ha) ENSO; 10 (1,143kg/ha) or 15% (1,430kg/ha) ANSE and the control (493kg/ha). However, the grain yield on those sprayed with 1:05v/v (1,969kg/ha) ENSO was statistically similar with those treated with 1:10v/v (1,704kg/ha) ENSO, 20% (1,729kg/ha) ANSE and mancozeb (2,033kg/ha). The control plants resulted in significantly lower grain yield than the treated plants.

Table 3. Effect of spraying varying concentrations of neem seed extracts on the yield components and grain yield of cowpea.

Neem Extract	Dosage	Number of pods/plant	Number of seeds/pod	Pod yield (kg/ha)	Grain yield (kg/ha)
NSO (l/ha)	2.0	9.5d	7.2bc	1766.0e	941.0e
	3.0	11.3b-d	9.3a	2039.0de	1315.0cd
	4.0	12.5a-c	9.7a	2239.0b-d	1469.0b-d
Mean		11.1	8.7	2015.0	1242.0
ENSO (v/v)	1:05	13.8ab	10.8a	2702.0a	1969.0a
	1:10	12.7a-c	10.2a	2521.0ab	1704.0ab
	1:15	11.7a-d	8.8ab	2213.0b-d	1505.0bc
Mean		12.7	9.9	2479.0	1726.0
ANSE (%)	10	10.0cd	9.0ab	1985.0de	1143.0de
	15	12.2a-d	10.0a	2155.0cd	1430.0b-d
	20	13.5ab	10.2	2463.0a-c	1729.0ab
Mean		11.9	9.7	2201.0	1434.0
Mancozeb (kg/ha)	2.5	14.2a	10.7a	2716.0a	2033.0a
Control	0	6.2e	6.2c	1194.0f	493.0f
Overall mean		11.6	9.3	2181.0	1430.0
SE±		0.66	0.46	78.79	82.02

NSO = neem seed oil; ENSO = emulsifiable neem seed oil; ANSE = aqueous neem seed extract

Means followed by the same letters within a column are statistically the same.

Discussions

Incidence

The study revealed that application of different concentrations of neem seed formulation has beneficial effects in the management of cowpea scab. The result depicted that application of 3.0l/ha NSO, 1:10v/v ENSO and 15% ANSE individually gave effective control of cowpea scab. Application of the neem seed extracts beyond these dosages did not differ significantly in the control of cowpea scab. This suggests that these dosages of the respective formulations are optimum for the control of cowpea scab under field conditions. It could therefore be asserted that the respective optimum dosages contains adequate active bio-principles consisting of azadirachtin, meliantriol and salanin, which act in concert with each other and apparently retard the growth and development of the disease on cowpea. The neem seed extracts provide a film coating layer over the sprayed cowpea parts, which act as a barrier between susceptible tissues and the pathogen, thus, protecting the cowpea tissues from penetration by the

pathogen. This consequently, deters conidial germination of dispersed spores on the plant parts and reduces number of tissues infected by the pathogen. This observation concur with the findings of Tijjani *et al.* (2014) and Dawakiji *et al.* (2016) who reported the fungicidal properties of neem seed extracts in the control of *Aspergillusflavus* and *Ralstonia solanacearum* on tomato and pepper respectively.

Severity

The severity of scab on different infected cowpea parts such as leaf, stem, peduncle and pod were significantly influenced by the application of different dosages of neem seed formulation. The severity of the disease was lower on plants treated with different concentrations of neem seed formulations than the control plant. The severity decreased with increase in the concentration of the neem formulations. This could be due to increase in active ingredients in the neem formulations, which were adequate enough to exert debilitating effect including disruption in normal physiological functions such as reproduction and growth of the pathogen. Among the infected cowpea parts, the disease was more severe on pods than leaves, stems and peduncles. The pods, being the organ for seed development, may contain high concentrations of assimilates for seed development and this may have provided some nutrients to the fungus for its growth and development. This observation agrees with the findings of Mungo *et al.*(1998) who used different cowpea parts as growth media for isolation of *Sphaceloma* sp. They obtained more isolates of the pathogen on pod extract agar than on leaf, stem or peduncle agar. This observation may be related to the possible availability of growth promoting factors for the fungus in the pod more than any of the other three plant parts. This partly explains the higher susceptibility of cowpea pods to scab than leaves, stems and peduncles.

Among the three neem seed formulations tested, ENSO was more effective in the control of scab than NSO or ANSE. Its superiority could be explained in line with the quantity and quality of the active principles present in it. During the preparation of ENSO, caustic soda (NaOH) was added which might have probably rendered the release of more active principles in the oil. The caustic soda has acted a synergy to the neem oil thereby improving effectiveness of ENSO in the management of cowpea scab than NSO and ANSE. This observation corroborates that of Saxena (1990) who reported that the efficacy of neem seed extracts could be enhanced by additives, synergist, antioxidant and improve methods of application. The efficacy of ENSO was comparable to that of mancozeb. An early finding by Locke, (1990) has shown the effectiveness of ENSO in the control of rusts and powdery mildew of ornamental plants.

Cowpea grain yield

Cowpea yield components and grain yield were higher on plants sprayed with different concentrations of neem seed formulations than the unsprayed plant serving as control. Plant sprayed with 3.0 or 4.0l/ha NSO; 1:05, 1:10 or 1:15v/v ENSO; and 15 or 20% ANSE produced more pods/plant, seeds/pod, pod and seed yield than the control. The yield components and seed yield increased with increase in concentration of the neem seed formulation. This is directly related to the disease intensity on the plants. The more severe the disease especially on pods the lower the yield. Young pods prior to grain filling were infected by scab and they become disfigured and mummified leading to pod abortion.

The pod mummification and/or abortion caused by scab is responsible for heavy yield losses in cowpea (Mungo *et al.,* 1995). Grain yield of plants treated with low dosages of neem seed formulation were low in quantity and quality due to the effect of the disease. The seeds were disfigured by the symptoms scab on their seed coat which reduced the quality. Thus, the plants that received adequate dosage of different neem seed formulations were less infected by scab thereby producing more good quality seeds than those that received low dosages. This observation corroborate with Mungo *et al.,* (1995) who reported low cowpea yield on plants treated with low

concentrations of benomyl than those treated with higher concentrations while controlling scab on cowpea.

Conclusion

From the results presented above, it is evident that there exist a potential in neem seed extracts in the control of cowpea scab disease.

Application of 1:05, 1:10v/v ENSO and 20% ANSE effectively reduced scab infection and increased grain yield of cowpea. The extracts if fully harnessed could be used as components of cowpea production packages.

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