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EVALUATION OF NEEM SEED EXTRACT AND FUNGICIDES (BENLATE AND APRON PLUS 50 DS) AS SEED DRESSING FOR THE MANAGEMENT OF FUNGAL LEAF SPOT DISEASES OF EGGPLANT

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ABSTRACT
Greenhouse and field experiments were conducted at the Faculty of Agriculture Research Farm, University of Nigeria, Nsukka during the 2010 growing season to evaluate the efficacy of neem seed extract (*Azadirachta indica* A. Juss) and fungicides (Apron plus 50Ds and Benlate), as seed dressing for the management of fungal leaf spot disease (*Cercospora melongenae*, CA) of eggplants (*Solanum melongena* L.). The experiments were laid out as a 4 x 3 factorial in Completely Randomized Design (CRD) and Randomized Complete Block Design (RCBD) for greenhouse and field experiments respectively. Result obtained showed that combination of neem seed extract treatment and black beauty eggplant variety were most effective in the management of fungal leaf spot disease of eggplant. Neem seed extract significantly (P=0.05) reduced number of leaf abscission, percentage aborted flowers and leaf spots disease incidences (spots/leaf and spots/plant) and severity, and also significantly (P=0.05) increased germination and survival counts 2 weeks after transplanting (WAT), leaf number per plant, number of fruits per plant, fruit weight and yield in both experiments. As regards to varietal effects, black beauty significantly (P=0.05) had reduced number of leaf abscission, flower abortion disease incidence and disease severity, and also significantly (P=0.05) increase survival counts 2 WAT, fruit weight per plant and yield. It was therefore recommended that neem seed extract be used as alternative to synthetic inorganic chemicals in combination with black beauty eggplant variety for the management of fungal diseases in eggplant.

KEYWORDS: *Azadirachta indica*, fungicides, seed dressing, fungal leaf spot disease management, eggplant

INTRODUCTION

Eggplant (*Solanum melongena* L.) is one of the most popular of the many species of solanum that bear non-sweet, non-acid vegetable type of fruit. The fruit is a fair source of cellulose, traces of protein and contains 89.6% water (Tindall, 1983). It is a nourishing and most welcome fruit vegetable eaten raw in formal and informal occasions by urban and rural Nigerians.

Seedling emergence, fruit yield and quality of the eggplant produced are reduced by various pathogenic organisms and pests, especially fungi. Fungicides of different chemical composition have been used in fungal diseases management of vegetables (Rice *et al.*, 1990; Tindall, 1983). However, numerous environmental and health problems had been reported to be associated with their use (FAO, 1983; Amadioha, 2000). Unguarded uses of these fungicides have also been reported to result in the development of disease resistance in many fungal species (Hewitt, 1998).

Neem plant (*Azadirachta indica* A. Juss) has been shown to be a potential alternative for the control of plant fungal diseases, with chemical compounds that are environmental friendly (Amadioha, 2000; Chiejina, 2006). Some constituents of the neem plant that effect diseases control demonstrate systematic action in certain plant species. Several tests have indicated considerable promise for use of neem plant in diseases management (Singh *et al*., 1980; Locke, 1990; Locke and Lawson, 1990; Amadioha, 2000; Okigbo and Emogheme, 2003; Chiejina, 2006). It is expected that the use of neem plant materials extract for diseases control as alternative to synthetic chemical will ensure safer plant produce and a healthier environment. The study was therefore conducted to examine in greenhouse and field experiments, the efficacy of neem seed extract and fungicides (Apron plus 50DS and Benlate) as seed dressing for management of fungal leaf spot disease of eggplant.

MATERIALS AND METHODS

Description of the study location

The experiments were conducted at the Faculty of Agriculture Research Farm, University of Nigeria, Nsukka during the 2010 growing season. The site is located within the latitude $06^{o}52^{l}$N, longitude $07^{o}24^{1}$E and at an altitude of 447.26 m above sea level. The area is characterized by distinct wet (April to October) and dry (November to March) seasons with rainfall bi-modally distributed, with peaks in July and September. The experimental field was under one year grass fallow. The soil of the area was characterized textually as a sandy loam. The clay and silt contents properties that enhance water retention were low, 24% and 20% respectively. The pH was low, as well as nitrogen and organic maters content. At the low soil pH, the aluminum content was high, while magnesium and calcium contents and base saturation were low (Table 1). There was need for soil amendment by use of nitrogen based fertilizer and organic manure.

Table 1. Soil Physical and Chemical Properties of the Experimental Site before Planting

Property	Content
Mechanical Properties	
Coarse Sand (%)	46.00
Fine Sand (%)	28.00
Clay (%)	24.00
Silt (%)	20.00
Textural Class	Sandy Loam
Chemical Properties	
pH (H_20)	4.30
pH (KCl)	3.70
Organic Carbon (%)	0.72
Organic Matter (%)	1.24
Total N (%)	0.0064
Available P (ppm)	8.80
Base saturation (%)	43.00
Exchangeable Cation (meg / 100g soil)	
Na	0.80
K	0.16
Ca	1.00
Mg	0.90
CEC (me / 100g)	7.00

Greenhouse Experiment

The greenhouse experiment was conducted as a 4 x 3 factorial laid out in a Completely Randomized Design (CRD). A total of 24 treatment combinations per replicate was used, and was replicated three times. Four varieties of eggplant seeds: Lokoja, Marvel, Black beauty and Edible leaves local were the experimental materials. Neem seed extract (3.0 ppm) and fungicides (Apron plus 50Ds and Benlate) were applied as seed dressing by soaking (Maude *et al*., 1969) before establishing the nursery. Active ingredients from grated mature neem seed kernels were extracted by ethanol extraction method (Kenneth, 1974). Concentration of Azadirachtin in parts per million (ppm) in each millilitre of extract was determined using the wavelength of the compound [217nm (E910) in ethanol (Znno *et al*., 1975)]. Seed Health Test was also carried out on the seeds (ISTA, 1993). Perforated polythene bags measuring 90cm x 90cm were used. Top soil from the same site of field experiment was used. Cured poultry manure was incorporated as basal dressing at the rate of 60 g/bag^{-1} 4 WBT and mulch with grass. Perforations on the bags ensured proper drainage. Soil in the bags was teased every two weeks to ensure proper aeration. Four week old seedlings from the nursery bed were transplanted, four per bag. Compound fertilizer (NPK: 20:10:10) was applied at the rate of 80 g/bag^{-1} as basal dressing 2 weeks after transplanting (WAT).

Field Experiment

The area was mowed, ploughed and harrowed for the study. A total land area of $310.3m^2$ was mapped out with $210.6m^2$ actually under plant cover. The experiment was conducted as a 4 X 3 factorial, laid out in Randomized Complete Block Design (RCBD). The treatment combinations were replicated three times. Each replicate was a block of 24 plots measuring 2m x 2m and 0.5m apart. Each plot contained twelve plants with 45cm x 60cm spacing within and between rows respectively, giving a total plant population of 288 plants per replicate and 864 plants per 210.6 m^2. Seed treatments were as in the greenhouse experiment. Cured poultry manure was worked into the plots as basal dressing at the rate of 120 kg/ha 4 WBT. Four week old seedlings from the nursery bed were transplanted into the plots and NPK compound fertilizer (20:10:10) at the rate of 150 kg/ha was applied two weeks after transplanting (WAT). No plant protectant was applied. Plots were manually weeded with hoe as the need arise.

Data Collection

Data were collected on some vegetative parameters, yield and yield characters of egg plant, and on the leaf spots disease incidence and severity.

Disease incidence and severity was scored using the scale of 0 – 3; with 0 = healthy (no leaf spot); 0.75 = mild infection (1 – 10 spots / leaf); 1.0 = moderate infection (11 – 20 spots / leaf); 2.0 = severe infection (21 – 40 spots/ leaf); and 3.0 = very severe infection (40 spots and above per leaf) (Cooke, 2006).

$$\text{Disease incidence } (I) = \frac{\text{Number of infected plant units}}{\text{Total number of plant units assessed}} \times 100$$

$$\text{Disease severity } (S) = \frac{\text{Area of diseased tissue}}{\text{Total tissue area}} \times 100$$

Data Analysis

Data collected were subjected to statistical analysis of variance (both greenhouse and field experiments) to test for the significance of treatment effects using GenStat 5.0 (2003).

RESULTS

Main Effects of Seed Treatments and Variety on Some Vegetative Parameters

Survival Counts at 2 WAT

Survival counts at 2 WAT showed significant (P=0.05) difference among the treatments applied and the varietal effects in the both experiment (Table 2). In the greenhouse experiment, *Azadirachta indica* seed extract treatment had the highest survival counts, when compared with the rest of the treatments. In the field experiment, Benlate had the highest survival counts, which was statistically similar to that of neem seed extract, but differed from the rest of the treatments. Black beauty consistently recorded highest survival counts in both the greenhouse and the field experiments, which differed from the rest of varieties.

Leaf Number per Plant at 50% Anthesis

The number of leaves per plant showed significant (P=0.05) difference among the treatments applied in the both experiment (Table 2). *Azadirachta indica* seed extract treatment had the highest leaf number per plant, which differed from the rest of the treatments. Varietal effect on the leaf number per plant also showed significant (P=0.5) differences (Table 2). In the greenhouse, Edible leaves local had the highest number of leaves per plant which differed from the rest of the varieties. In the field experiment, on the other hand, Black beauty recorded highest number of leaves per plant and it different from the rest of the varieties.

Plant Height (cm) at 50% Anthesis

The result in Table 2 showed that plant height at 50% anthesis differed significantly at 5% level of probability among the treatment and the varieties in the both experiments. In the greenhouse experiment, Apron plus 50Ds produced the tallest plants which showed statistical similarity with that of Benlate, but differed from the the rest. In the field experiment, *Azadirachta indica* seed extract treatment produced plants with the tallest height and it differed from the rest of the treatments. Varietal effect on the plant height at 50% anthesis also showed significant (P=0.5) differences as shown in Table 2. In the both experiments, Edible leaves local produced the tallest plant height which differed statistically from the rest of the varieties, in the both experiments

Number of Leaf Abscission at 50% Anthesis

The result showed that there were significant (P=0.05) differences in the number of leaf abscission in the seed treatments applied and varietal effects (Table 2). Neem seed extract reduced number of leaf abscission per plant at 50% anthesis when compared to the other treatments in the greenhouse and field experiments. Varietal effect was also significant (P=0.05) as shown in Table 2. Black beauty variety recorded the lowest leaf abscission in both the greenhouse and the field experiments, and was different from the rest of the varieties.

Table 2. Main Effects of Seed Treatments and Variety on Some Vegetative Parameters

	Greenhouse Experiment				Field Experiment			
Treatment	Survival Counts 2 WAT (%)	Leaf No. per Plant at 50% Anthesis	Plant Height (cm) at 50% Anthesis	No. of Leaf Abscission 50% Anthesis	Survival Counts 2 WAT (%)	Leaf No. per Plant at 50% Anthesis	Plant Height (cm) at 50% Anthesis	No. of Leaf Abscission 50% Anthesis
Neem seed extract (3.0 ppm)	93.42a	54.25a	42.17b	2.75a	84.92ab	52.83a	46.73a	9.00 a
Apron Plus 50Ds	88.67b	44.25b	44.42a	5.25c	84.33b	46.25c	46.31b	9. 67b
Benlate	87.00c	37.00c	44.16a	4.25b	85.42a	48.17b	43.36c	9.00a
Control	84.67d	29.92d	41.94b	6.50d	70.42c	26.75d	40.98d	12.25c
Mean								
F-LSD $_{(0.05)}$	1.077	0.291	0.913	0.821	0.840	0.610	0.372	0.097
Variety								
Lokoja	89.17b	46.67c	47.11b	4.33b	77.11d	41.17c	48.67b	8.11b
Marvel	88.50b	49.83b	33.73c	4.67b	78.17c	58.00a	18.68d	10.17c
Black Beauty	93.67a	27.92d	29.91d	3.00a	86.22a	30.72d	38.69c	3.50a
Edible leaves local	87.11c	55.06a	66.04a	4.50b	84.67b	51.17b	74.07a	12.50d
Mean								
F-LSD $_{(0.05)}$	0.880	0.237	0.745	0.670	0.686	0.498	0.304	0.079

Table 3. Main Effects of Seed Treatments and Variety on some Yield and Yield Components

	Greenhouse Experiment				Field Experiment			
Treatment	Flower Abortion (%)	No. of Fruits per Plant	Fruit weight per plant (gm/plant)	Yield (t/ha)	Flower Abortion (%)	No. of Fruits per Plant	Fruit weight per plant (kg/plant)	Yield (t/ha)
Neem seed extract (3.0 ppm)	18.00a	49.70a	200.26a	5.615a	17.67a	45.53a	222.19a	5.563a
Apron Plus 50Ds	24.50b	42.34b	162.77b	5.140b	37.58b	40.15b	171.21b	5.090b
Benlate	31.00c	39.79c	148.57c	4.999c	37.67b	37.05c	147.00c	4.798c
Control	42.00d	32.98d	123.53d	4.756d	50.00c	31.12d	139.22c	4.510d
F-LSD $_{(0.05)}$	0.821	0.797	1.043	0.2664	0.480	0.283	15.132	0.0035
Variety								
Lokoja	32.50d	46.31c	134.19c	4.553b	38.00d	41.59c	120.94c	4.475b
Marvel	31.33c	50.80b	169.07b	4.280c	35.78c	47.29b	227.87b	4.008c
Black Beauty	18.17a	7.69d	304.22a	8.304a	25.72a	7.13d	265.48a	8.281a
Edible leaves local	21.67b	70.26a	77.05d	3.875d	31.33b	65.33a	74.35d	3.820d
F-LSD $_{(0.05)}$	0.670	0.650	0.852	0.2175	0.392	0.231	44.004	0.0028

Table 4. Main effect of seed treatment on leaf spot disease symptoms of eggplant under greenhouse and field conditions

Treatment	Greenhouse Experiment			Field Experiment		
	No. of spots per leaf	No. of spotted leaves per plant	Disease (Severity) Index (%)	No. of spots per leaf	No. of spotted leaves per plant	Disease (Severity) Index (%)
Neem seed extract (3.0 ppm)	3.50a	3.08a	5.55a	13.83a	9.83a	10.92a
Benlate	4.00ab	4.42b	5.55a	18.00c	12.58c	11.68a
Apron Plus 50Ds	4.75b	5.25c	5.20a	16.33b	11.08b	13.35b
Control	6.75c	7.50d	10.76b	22.75d	15.67d	23.86c
F-LSD $_{(0.05)}$	0.821	0.815	1.454	0.449	0.134	1.328
Variety						
Lokoja	3.67a	4.83b	7.17b	19.44c	12.17b	15.25b
Marvel	4.50b	5.17c	6.94b	17.28b	13.28c	15.24b
Black Beauty	3.33a	2.67a	5.55a	6.33a	6.33a	7.94a
Edible leaves local	5.67c	5.67c	7.17b	22.94d	15.61d	20.06c
F-LSD $_{(0.05)}$	0.670	0.666	1.188	0.367	0.109	0.268

Main Effects of Seed Treatments and Variety on some Yield and Yield Components

Flowers abortion
Different seed treatments and varieties of eggplant showed significant (P=0.05) reduction in the number of aborted flowers at 50% fruit set (Table 3). Neem seed extract had the least number of aborted flowers at 50% fruit set than Apron plus 50Ds, Benlate and control in both experiments. The control recorded highest flowers abortion and was severe in the field than in the greenhouse experiment. Black beauty variety recorded lowest flower abortion in the both experiments when compared with the rest of the varieties. Black beauty variety also had higher number of aborted flowers in the field than in the greenhouse experiments.

Number of fruits per plant at harvest
Results in Table 3 showed significant (P=0.05) differences in seed treatments and variety on the number of fruits produced per plant. The neem seed extract had the highest number of fruits per plant at harvest when compared with Apron plus 50Ds, Benlate and control in that order, and in the both experiments. Edible leaves local variety produced highest number of fruits per plant in the both experiments, and it differed from the rest of the varieties.

Fruit weight per plant (gm/plant)
The result showed that fruit weight per plant differed significantly at 5% level of probability among the treatments and variety in the both experiments (Table 3). *Azadirachta indica* seed extract treatments in both greenhouse and field experiments produced plant with the highest fruit weight which differed statistical from the rest of the treatments.

As regards the varietal effects, in the both experiments, Black beauty produced plant that had the highest fruit weight which differed statistically from the rest of the varieties.

Yield (t/ha)
Results in Table 3 showed significant (P=0.05) differences in seed treatments and variety on the yield (t/ha). The neem seed extract had the highest yield when compared with Apron plus 50Ds, Benlate and control in that order, and in the both experiments. Black beauty variety produced highest fruit yield per hectare in the both experiments, and was different from the rest of the varieties.

Main Effect of Seed Treatment on Leaf Spot Disease Incidence and Severity of Eggplant under Greenhouse and Field Conditions

Number of spots per leaf
The result in Table 4 showed that number of spots per plant differed statistically at 5% level of probability among the treatments and variety in the greenhouse and field experiments. *Azadirachta indica* seed extract treatments in the both experiments produced plants with the least number of leaf spots. In the greenhouse experiment, it showed statistical similarity with that of Benlate and differed from the other treatments. In the field experiment, the neem seed treatment differed from the rest of the other seed treatments. As regards the varietal effects, in the both experiments, Black beauty produced plant that had the least number of leaf spots which differed from the rest of the varieties.

Number of spotted leaves per plant
The result showed that number of spotted leaves per plant differed significantly at 5% level of probability among the seed treatments, and variety in the both greenhouse and field experiments (Table 4). *Azadirachta indica* seed extract treatments in the both experiments produced plants with the least number of spotted leaves per plant, which differed from the rest of the treatments. Black beauty in the both experiments produced plants with least number of spotted leaves which differed statistically from the rest of the varieties.

Disease (Severity) Index (%)
Results in Table 4 showed that there were significant (P=0.05) differences among the seed treatments and variety on disease index (%) in both the greenhouse and field experiments. In the greenhouse experiment, neem seed extract and Benlate had the least disease index and they showed statistical similarity with that of Apron plus 50Ds. The control recorded highest disease index and was severe in the field than in the greenhouse experiment. Black beauty variety recorded lowest disease index in the both experiments when compared with the rest of the varieties. Black beauty variety also had higher disease index in the field when compared with that of greenhouse experiment.

DISCUSSION

Results from this study indicated that neem seed extract possesses active ingredients which exhibit fungitoxic action. This is in agreement with reports of different workers (Singh *et al*., 1980; Locke, 1990; Locke and Lawson, 1990; Amadioha, 2000; Okigbo and Emogheme, 2003; Chiejina, 2006). It is therefore not surprising in the present study that neem seed extract increased survival counts of seedlings and maintained plant population till harvest as against the control. Number of flowers aborted at 50% fruit set was reduced which reflected in increased number of fruits per plant at harvest. Leaf abscission and leaf diseases such as leaf spots represent an indirect loss in yield. Leaves provide surface areas for the production of assimilate for fruits filling. Seed extract effectively reduced leaf abscission and leaf spots disease severity which was positively reflected in fruits weight and yield at harvest.

The performance of neem seed extract in these experiments compared effectively with other standard synthetic fungicides used in plant diseases management such as Apron plus 50Ds and Benlate and much better sometimes. For instance, Singh *et al.* (1980) had reported that neem seed oil is effective against *Rhizoctonia solani* and *Scleratinia sclerotiorum* and also powdery mildew (Locke, 1990) where it gave 100% control on hydrangeas in greenhouse better than Benlate, the standard mildew treatment.

The increased effect of neem seed extract in the greenhouse compared to the field environment as was observed could be caused by temperature, soil pH and other vagaries of weather. The greenhouse environment is more stable in terms of weather changes. Locke and Lawson (1990) had reported that activity of neem plant materials is affected by temperature, soil pH and other changes in weather. However, the presence of active components in plants is influenced by several factors such as method of extraction, age of plant, time of harvesting plant materials and different extracting solvents. These also could have contributed to affect the activity of the neem seed extract.

Neem seed extract as used in this study was preventive rather than curative. This mean that farmers could have diseases prevented in advance thereby increase yield. Amadioha (2000) had reported that the greatest reduction in the incidences and severity of blast disease was observed in plants treated with neem plant extracts 2 days before inoculation with *Pyrecularia oryzae*, suggesting that the extract would be more effective when applied preventively as opposed to curatively. Locke and Lawson (1990) also reported that neem plant materials gave 90% control against bean rust when applied before the plants were exposed to the fungus. It worked poorly once rust was established.

Neem plant materials have been reported to be non-phytotoxic, non-toxic to humans and animals, friendly on non-target organisms and insects in farms, and above all bio-degradable and easily available to the farmers. This eliminates cost and other problems associated with the use of synthetic fungicides in plant disease control. It is reasonable to conclude therefore, that neem plant extracts, especially the seed extract could be suitable alternatives to fungicides in the management of fungal leaf spot of eggplant in the field.

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