

EFFECT OF PRE-AND-POST GERMINATION TREATMENTS OF SOYBEAN (*Glycine max*.L) WITH AQUEOUS EXTRACT OF MORINGA OLEIFERA STEM BARK ON FUNGAL INFECTION

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ABSTRACT

Experiments were conducted to evaluate the effects of pre-and-post germination treatments of soybean with aqueous extract of *Moringa oleifera* stem bark on seed-borne fungi infection. To test the pre-germination effect of the treatment, in-vitro experiments were first conducted to isolate and identify the seed-borne fungal pathogens in the soybean cultivar (TGX 1448). The seeds were soaked for 3 hours at 5 levels (1%w/v, 5%w/v, 10%w/v, 15%w/v, 20%w/v) of aqueous *Moringa oleifera* bark extract. Carbendazim fungicide and sterile distilled water (applied at 0.3% of seed weight) served as positive and negative controls respectively. The seeds were then plated on Potato Dextrose Agar to isolate the fungus and pure cultures prepared from the isolates. A potted experiment was further performed to test the efficacy of aqueous *Moringa oleifera* stem bark extract as pesticide in ameliorating the pathogenic effects of the fungal pathogens on soybean cultivar TGX 1448. Three seeds were sown per 10-litre (50cm diameter) plastic pots filled with steam sterilized sandy-loam soil. Aqueous *Moringa oleifera* bark extract (15g/litre) was used as pesticidal application on emerging leaves from the 2nd to the 8th week after planting. The results revealed that five (5) fungal isolates to be seed borne in the soybean variety; the use of the extract at the pre and post-germination stages of seed growth suppressed fungal growth and disease severity. Aqueous extract of *Moringa oleifera* stem bark is a potent alternative to inorganic chemicals as fungicide in soybean cultivation.

Keywords: *Moringa oleifera*, *Glycine max*., Fungal isolate, Fungicide, Botanical pesticide.

INTRODUCTION

Soybean is one of the important sources of oil and protein in the world and it is commonly used in both human and animal diets (Ariyo, 1995). It is not an indigenous crop in Nigeria, but is however gaining popularity in the country because of its numerous potentials that rank it even better than cowpea in the supply of high quality protein (Atungwa and Afolabi, 2001). Soybean has the potential of improving human diet through the supply of high quality protein as well as animal

feed and serves as a source of raw material base for agro-industries. Nigeria is the largest producer of soybean for food in West and Central Africa (Root *et al.*, 1987). Soybean diseases normally result in major yield loss in Nigeria. Some of the common diseases are caused by fungi, bacteria and viruses. Soybean rust, caused by *Phakopsora pachyrhizi*, is one of the most important foliar diseases in Nigeria. The infected leaves have small tan to dark brown or reddish brown lesions on which small raised pustules occur on the lower surface of the leaves (IITA, 2009).

A number of soybeans fungal pathogens are seed-borne in nature and may be transmitted to seedlings raised from the seeds when they are used as buffer stocks (Shovan *et al.*, 2008). The association of fungi with the seeds of soybeans can cause reduction in germination and result to reduced quality and quantity of soybean harvest (Mayongo and Kapooria, 2003).

Most of the synthetic chemical pesticides cause serious negative impacts, such as harmful effects on non-target beneficial organisms and unbalanced ecosystem due to pollution of soil, water and environment (Gupta and Shyam, 1996). Oruonye and Okrikata (2010), suggested botanicals as economically effective and environmentally friendly alternative to synthetic pesticide use in laboratory and field crop protection.

Moringa oleifera is a tropical multipurpose tree that naturally grows in India, Sub Saharan Africa and South America (Jahn, 1988). Almost every part of the plants (leaves, flowers, seeds, roots and bark) can be used as food or for medicinal and therapeutic purposes. *M. oleifera* bark and branches are not as useful in culinary uses as the leaves, seeds, fruit and flowers. The bark of *Moringa oleifera*, like its roots, possesses concentrated antibiotic properties (Anwar *et al.*, 2007).

The objectives of the study were to isolate and identify seed-borne fungal pathogens associated with the widely grown soybean cultivar (TGX 1448) in Kwara State and evaluate the efficacy of different concentrations of aqueous extract of *Moringa oleifera* stem bark on fungal growth and disease severity in the soybean variety.

MATERIALS AND METHODS

The experiments were carried out at the Crop Protection Research Laboratory and at the pavilion of the Crop protection Department of the Faculty of Agriculture, University of Ilorin, Kwara State-Nigeria. The soybean cultivar TGX 1448 was obtained from International Institute of Tropical Agriculture (IITA) Ibadan.

Laboratory experiment

The soybeans seeds used were surface sterilized using 1% sodium hypochlorite (NaOCl) for 30 seconds and later rinsed in several changes of sterile water. Ten soybean seeds each, were then soaked for 3 hours at 5 levels (1%w/v, 5%w/v, 10%w/v, 15%w/v, 20%w/v) of aqueous extract of *Moringa* stem bark, while

Carbendazim fungicide and sterile distilled water (applied at 0.3% of seed weight), served as positive and negative controls respectively.

To isolate the fungi, the Agar (PDA) plate method as described by the ISTA, (1996) was adopted. Hyphae and spores were transferred aseptically unto fresh sterile media with ethanol flamed inoculating needle to prepare the pure cultures from each of the fungal isolate. The plates were later incubated for seven (7) days at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The slides were observed under a compound microscope and the microscopic features recorded for each isolate. These features were then matched with standard references used for identification of the fungal isolates according to Mathur and Kongsdal, (2003). The diameter of the fungal isolates were measured at 11 days after treatment with the extract, following two perpendicular lines passing through the centre of the organism.

Layout of potted experiment and preparation of *Moringa oleifera* bark aqueous extract

Three soybean seeds were sown per 10-litre (50 cm diameter) plastic pot filled with sandy-loam soil, steam-sterilised at 121°C for 1 hour. The plants were thinned down to two per pot 7 days after germination. Each of the treatments were replicated thrice and this gave a total of 36 plastic pots.

The stem bark of *Moringa oleifera* plant was chopped into smaller sizes and 15 grammes of which was soaked in 1 litre of water for 24 hours. The extract was then sieved out with a muslin cloth. The extract was always prepared at the time of application as fungicide and the first application was at 2 weeks after planting and this continued on a weekly basis until the 8th week after planting.

Data collection and analysis

Data were collected in the in-vitro experiment on the occurrence, identification and effect of the extract on fungal growth. Percentage inhibition of the fungal growths was calculated from the result viz;

$$\text{Percentage inhibition} = \frac{dt - de}{dt} \times 100\%$$

Where:

dt (cm) = diameter of fungal growth in petri-dishes with un-amended medium,

de (cm) = diameter of fungal growth in petri-dishes with plant extract amended medium (Wokecha and Okereke, 2005).

In the potted experiment, weekly data were collected on each treatment for 9 weeks for the following parameters: Plant height (using a meter rule), number of leaves and the number of leaves manifesting fungal symptoms and the dry weights of pods and

grain at harvest. Percentage severity was calculated as the percentage of leaf on the plant with fungal disease symptoms.

All collected data were subjected to analysis of variance using the Statistical Package for Social Scientists (SPSS version 15) in order to ascertain significant differences determined at 5% using the New Duncan Multiple Range Test. (Duncan, 1955)

RESULTS

(A) *IN-VITRO* EXPERIMENT

(i) Occurrence and Identification of Fungi

Based on the observed morphology and microscopic characteristics, five fungal isolates were isolated and identified from the soybean seeds (Table 1). These are:

- (a) *Aspergillus flavus*
- (b) *Aspergillus niger*
- (c) *Rhizopus arrhizus*
- (d) *Saccharomyces cerevisiae*
- (e) *Fusarium oxysporum*

(ii) *In-vitro* effect of extract on fungal growth

The extract significantly inhibited fungal growth in all five isolated fungi in comparison to the negative control (Table 2). The effect of 10g/liter of the extract on the isolates showed that the significantly lowest mycelia growth was in *Aspergillus flavus* (3.5cm). In the case of the fungal isolates of *Aspergillus niger*, *Rhizopus arrhizus*, *Saccharomyces cerevisiae* and *Fusarium oxysporum*, the treatment of seeds with aqueous extract of 15g/liter significantly suppressed fungal growth to mean values of 3.8cm, 6.4cm, 13.3cm and 0.4cm respectively. This indicates that although all the treatments showed some fungicidal properties (compared with negative control), the rate of 15g/liter seems to be the most effective of all the concentrations except for 10g/l treatment of *A. flavus* when compared with the positive control.

(B) POTTED EXPERIMENT

(i) Disease severity

Table 3 shows the effect of time of application of treatment on percentage disease severity from 2nd to 9th week after germination (WAG). The Table indicates that at the 4WAG, there were no significant differences in disease severity between application of the treatment at the 3rd, 4th, 5th and 6th week after planting (WAP) but treatment application at 2WAP had the significantly lowest disease severity (1.8%). At the 5WAG, the disease severity was significantly less at the application of the treatment at 2WAP (3.4%) and 6WAP (3.9%). By the 6WAG, the lowest disease

severity was recorded at an application of extract at 2WAP (6.2%). This observable trend continued till the 9th week of data collection.

(ii) Number of leaves

Table 4 shows the effect of different treatment on number of leaves over 9 weeks after germination. The table shows that at 2WAG application of treatment at 2WAP produced the significantly highest number of leaves (7.0) compared with the other treatments which values were not significantly different. By the 4th week of data collection the lowest number of leaves were recorded at 6WAP (5.7), compared with the application at 2WAP, which had the significantly highest number of leaves (8.0). This observable trend continued to the 9th week after germination.

(iii) Plant height

The effect of the application of aqueous extract of Moringa on plant height (Table 5), shows that the treatments significantly influenced plant growth. The significantly tallest plants at 2WAG was at the treatment of 4WAP (11.5cm), 5WAP (11.4cm) and 6 WAP (11.4cm). This non-uniform trend continued to the 9th week of data collection with the tallest plants being the control (53.3cm).

(iv) Yield parameters

Table 6 shows the effect of the treatments on number of seeds per pod and weight of seeds per pod. The results indicated that the significantly highest number of seeds per pod and weight of seeds per pod were 2.2 and 14.2g respectively, obtained with an application of the extract on soybean plant at two weeks after planting.

DISCUSSION

According to Yang (2002), many destructive pathogens of soybeans are seed borne and the infected soybean seeds can reduce seed viability and if such seeds are sown for production of subsequent crops can cause reduced yields. Fungi of the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizoctonia* are commonly known to produce toxic substances, such as aflatoxin B1 and B2, Aspergellie acid, cyclopiezonic acid, kojic acid, naphthoquinones, fumonizin and fusaric acid. The substances are discharged by fungi in their metabolic processes (Singh *et al.*, 1991) and cause reduction in seed germination (Ibraheem, *et al.*, 1987). The present study however, showed only five fungi to be in association with the soybean seeds. This finding is similar to previous report of Uwala and Ekpe (2000) that also reported similar organisms from the seeds of soybean.

The result of the in-vitro experiment showed the inhibitory properties of aqueous Moringa extract on soybean seeds at different concentrations. Bandopadhyay (2002), studied the action mechanism of neem by-products on vegetative growth and reproduction of fungi and stated that, phytoalexins, sulfurade compounds and triterpenoids in these products have fungitoxic action and reduced mycelial growth.

It could therefore also be deduced from the present study that the fungistatic ability of the extract on the seed-borne fungi was probably due to the presence compounds such as phytoalexins, sulfurade and triterpenoids in the Moringa bark extract used for seed treatment.

The potted experiment revealed the use of aqueous Moringa extract as pesticide to be equally effective, which resulted in higher plant growth and yield parameters. Yusuf *et al.* (1998) observed powders of leaves of *Azadiracta indica*, *Melia azadirach*, *Zingiber officinale*, *Eucalyptus camaldulensis*, *Ocimum basilicum*, *Capsicum frutescens* and wood ash of *Khaya senegalensis* to be effective in the control of maize weevil (*Sitophilus zeamais*) in stored maize. The potential of botanical pesticides is characterized by low mammalian toxicity, lack of mutagenic activity and high rate of biodegradability (Kloss and McCullough, 1987). The result is in agreement with the finding of Satish (2007), that biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies.

On the basis of the results obtained it can be concluded that the use of aqueous Moringa extract caused a reduction in the occurrence and severity of fungal diseases in soybean cultivar (TGX 1448) and favourably compared with the inorganic synthetic fungicide used. This indicates that aqueous Moringa extract could serve as an effective alternative to the use of fungicide in the control of fungal diseases in soybean.

Table 1: Fungal isolates from soybean used for the experiment

Designation of Isolate	Growth characteristic on plates	Probable identification
A	Light green massively sporulating at the top. Cream colored reverse.	<i>Aspergillus flavus</i>
B	Black sporulating at the top. Cream colored reverse.	<i>Aspergillus niger</i>
C	Massive delicate mycelia with evidence of sporangia	<i>Rhizopus arrhizus</i>
D	Brownish slightly raised smooth colonies.	<i>Saccharomyces cerevisiae</i>
E	White cottony raised mycelia at the top and cream colored reverse.	<i>Fusarium oxysporum</i>

Table 2: Effects of the different *Moringa oleifera* bark extracts on specific fungal growth

Treatment	Organism				
	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Rhizopus arrhizus</i>	<i>Saccharomyces cerevisiae</i>	<i>Fusarium oxysporum</i>
1g/L	10.0 ^d	5.3 ^c	13.3 ^a	20.9 ^a	3.4 ^b
5g/L	8.7 ^a	15.3 ^b	9.7 ^b	16.8 ^a	3.4 ^b
10g/L	3.3 ^a	16.7 ^b	8.9 ^b	20.0 ^a	3.3 ^b
15g/L	6.4 ^b	3.8 ^b	6.4 ^b	13.3 ^a	0.4 ^a
20g/L	10.9 ^b	7.7 ^d	10.0 ^b	17.7 ^{cd}	6.7 ^a
CZ	3.1 ^a	0.8 ^a	0 ^a	3.3 ^a	0 ^a
SDW	23.3 ^d	26.7 ^a	20.0 ^c	26.7 ^a	8.6 ^a
Std err	0.352	0.442	0.352	0.436	0.632

Key: CZ = Carbendazim, SDW = Sterile distilled water, Std err = Standard error. Values with different superscripts within a column (in each segment) are statistically different at $P < 0.05$.

Table 3: The effects of the different *Moringa oleifera* bark extracts on percentage disease severity

Treatment	Weeks after germination / % disease severity							
	2WAG	3WAG	4WAG	5WAG	6WAG	7WAG	8WAG	9WAG
2 WAP	0	0	1.8 ^b	3.4 ^a	6.2 ^c	6.4 ^b	9.7 ^d	10.9 ^c
3 WAP	0	0	2.8 ^a	6.5 ^b	7.7 ^a	12.4 ^b	15.7 ^{ab}	16.8 ^a
4 WAP	0	0	3.2 ^a	7.7 ^a	7.6 ^a	10.9 ^b	15.1 ^{abc}	16.3 ^a
5 WAP	0	0	2.8 ^a	6.2 ^a	7.9 ^a	11.5 ^b	16.2 ^{ab}	17.7 ^a
6 WAP	0	0	3.1 ^a	3.9 ^a	8.8 ^a	11.1 ^b	14.6 ^{bc}	16.3 ^a
CZ	0	0	0.7 ^a	3.9 ^a	4.6 ^a	6.5 ^b	14.0 ^{bc}	14.9 ^b
SDW	0	0	2.7 ^{ab}	6.8 ^{ab}	9.2 ^b	11.4 ^b	8.2 ^a	9.7 ^a
Std err	0.00	0.00	0.443	0.487	0.345	0.704	0.563	0.558

Key: CZ = Carbendazim, SDW = Sterile distilled water, Std err = Standard error, WAP = week after planting, WAG = week after germination. Values with different superscripts within a column (in each segment) are statistically different at $P < 0.05$.

Table 4: The effects of the different *Moringa oleifera* bark extracts treatment on number of leaves over 9 week period

Treatment	Weeks after germination / Number of leaves							
	2WAG	3WAG	4WAG	5WAG	6WAG	7WAG	8WAG	9WAG
2 WAP	1.2 ^b	7.0 ^a	8.0 ^b	11.5 ^c	15.6 ^b	18.9 ^b	23.5 ^b	25.9 ^b
3 WAP	3.3 ^b	4.0 ^a	7.3 ^{cd}	9.9 ^{cd}	13.5 ^a	15.3 ^a	20.0 ^d	22.2 ^d
4 WAP	3.2 ^b	3.7 ^a	6.5 ^a	9.7 ^a	13.9 ^a	15.7 ^a	20.2 ^d	21.9 ^d
5 WAP	3.3 ^b	4.0 ^a	6.9 ^d	9.2 ^b	13.5 ^a	16.9 ^a	21.6 ^c	24.0 ^c
6 WAP	3.2 ^b	4.0 ^a	5.7 ^a	8.7 ^a	12.7 ^a	15.0 ^a	19.1 ^d	21.1 ^c
CZ	3.0 ^b	5.3 ^b	9.3 ^a	13.6 ^b	18.3 ^b	21.0 ^a	26.0 ^b	28.3 ^a
SDW	3.2 ^b	4.2 ^a	7.0 ^b	10.4 ^a	13.7 ^a	16.9 ^a	21.9 ^b	23.8 ^a
Std err	0.244	0.275	0.493	0.364	0.313	0.323	0.553	0.633

Values with different superscripts within a column (in each segment) are statistically different at $P < 0.05$.

Table 5: The effect of the different *Moringa oleifera* bark extract treatment on plant height over 9 week period

Treatment	Weeks after germination / Plant height							
	2WAG	3WAG	4WAG	5WAG	6WAG	7WAG	8WAG	9WAG
2 WAP	10.8 ^a	16.2 ^b	22.1 ^b	38.3 ^b	36.8 ^c	41.7 ^c	47.2 ^b	49.4 ^b
3 WAP	10.2 ^a	15.1 ^b	19.3 ^b	24.9 ^b	32.4 ^{abc}	39.0 ^c	44.0 ^c	46.1 ^c
4 WAP	11.5 ^b	14.6 ^{bc}	18.8 ^b	22.5 ^b	32.1 ^{bc}	33.6 ^d	37.9 ^c	40.1 ^d
5 WAP	11.2 ^b	13.3 ^a	17.4 ^a	22.9 ^{ab}	30.7 ^c	33.9 ^d	37.6 ^c	39.4 ^d
6 WAP	11.3 ^b	12.8 ^a	15.9 ^a	19.8 ^a	25.9 ^b	29.8 ^c	33.9 ^a	36.2 ^a
CZ	10.8 ^a	13.6 ^{ab}	23.2 ^b	30.5 ^a	38.7 ^a	45.2 ^a	50.3 ^a	53.3 ^a
SDW	10.3 ^a	18.6 ^a	19.5 ^a	26.2 ^a	33.6 ^a	43.5 ^b	47.2 ^a	50.3 ^b
Std err	0.512	0.506	0.576	0.743	0.996	0.867	0.934	0.921

Values with different superscripts within a column (in each segment) are statistically different at $P < 0.05$.

Table 6: Effect of Treatment on Yield Parameters at Harvest

Treatment	Number of seeds/pod	Weight of seeds/pod(g)
2 WAP	2.2 ^a	14.2 ^a
3 WAP	1.6 ^a	13.6 ^a
4 WAP	1.4 ^a	10.9 ^a
5 WAP	1.0 ^a	9.7 ^a
6 WAP	0.8 ^a	7.8 ^a
CZ	3.6 ^a	16.1 ^a
SDW	1.1 ^a	10.0 ^a
Std err	0.133	0.412

Values with different superscripts within a column (in each segment) are statistically different at $P < 0.05$.

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