Research in Plant Biology, 4(1): 01-11, 2014 ISSN : 2231-5101
www.resplantbiol.com

Regular Article

Interactive effects of some botanicals and *Fusarium* spp. on the growth of millet seedlings

***[1]Akanmu Akinlolu Olalekan, [1]Olawuyi Odunayo Joseph, [1]Abiala Moses Akindele, [2]Yaya Olaoluwa Simon, [1]Odebode Adegboyega Christopher**

[1]Department of Botany, University of Ibadan, PMB 128, Ibadan, Nigeria
[2]Department of Statistics, University of Ibadan, Ibadan, Nigeria
*Corresponding author: akinakanmu@gmail.com

Millets are economically important cereal crop with high protein contents that benefits man and animals. However, the cultivation of this cereal in Southwestern Nigeria is greatly affected by some soil borne pathogens, of which *Fusarium* species has been reported. Hence, this study investigated the interactive effects of some pathogenic *Fusarium* species (*F. anthophilum, F. scirpi, F. verticillioides* and *F. oxysporum*) and aqueous botanicals on the growth of millet seedlings. *In vitro* and *in vivo* studies of *Mangifera indica* and *Jatropha caucas* (0.15, 0.30 and 0.45 mg/ml concentrations), *Moringa oleifera*, *Manihot esculenta* and *Senna alata* (0.05, 0.10 and 0.15 g/ml concentrations) were conducted. The *Extracts* and *Concentration levels*, *Fusarium* species, as well as *time* of observation, recorded significant ($p<0.05$) effects with respect to the millet seedlings growth. However, high significant ($p<0.01$) interactions were obtained from *Extracts x Fusarium* species, *Extracts x Concentration levels, Concentration levels x Time of observation* and *Extracts x Concentration levels x Time of observation.* The order of efficacy of the extracts showed that; *J. curcas* > *M. indica* and *M. oleifera* > *M. esculenta* > *S. alata* increases with concentration levels and time of observation, thus, significantly ($p<0.05$) antagonized the pathogenic effect of the *Fusarium* species. This study therefore, provides valuable information on the interactive treatments that best enhances the optimum performance of the botanicals against the pathogenic *Fusarium* species of millet seedlings.

Keywords: Millets, *Fusarium* species, Plant extracts, Interactions.

Millet is a cereal with good drought tolerance and hardiness widely grown in the hot and dry climates areas of arid and semi-arid regions of Africa and southern Asia (Mohammed *et al*. 2012). It is one of the four most important cereals crop (millets, sorghum, maize and rice) normally grown where rain fall is not sufficient (200-600 mm) for corn and sorghum (Singh *et al*. 2010). The crop which has diverse varieties (Andrews and Kumar 1992) is produced primarily for human consumption as staple food (78%) with other uses of less than 20%, while its use in countries like Mexico, Australia, Canada and the United State of America is mainly for livestock feeds (Obilana 2003). However, millet crops remain the key source of food security and energy for about 250 million people in sub-Saharan Africa, where it has contributed significantly to the socio-economic, food, shelter, health and environment of the inhabitant poor people (Dicko *et al*. 2005; Gari 2001; Mohammed *et al*. 2012). *Fusarium*

species are soilborne pathogenic fungi known to be associated with most cereal crops. The ability of these species to cause disease at all stages of plant development have been reported on cereals which include; maize, sorghum, wheat, barley (Kedera *et al.* 1992; Visconti and Doko 1994; Soonthornpoct *et al.* 2000). The pathogenic activities of *Fusarium* species causing diseseas on the stem, root, stalk and ear of most cereals had been reported earlier on maize and millet (Visconti and Doko 1994; Leslie 2005), while *F. anthophilum, F. verticillioides, F. oxysporum* and *F. scirpi* were the most pathogenic *Fusarium* species of millet in southwestern Nigeria (Akanmu *et al*. 2013a). Thus, this pathogen posed imminent threat to the sustainable productions of this cereal, hence, the need for the disease control. The biological control measures using plant extracts offer a vast, virtually untapped reservoir of chemical compounds with many potential (Scott *et al*. 2004). The plant extracts is of more importance in agriculture to manage various plant diseases with less risk compared to synthetic compounds that are toxicologically and environmentally undesirable, Therefore, there are increased attention paid to natural products since the past decade (Duke *et al*. 2003). The search for new bioactive compounds for a wide range of modes of action necessitated the need to investigate the *in vitro* and *in vivo* interactive effects of the plant extracts; *Jatropha caucas, Mangifera indica, Moringa oleifera, Manihot esculenta* and *Senna alata* against the pathogenic *Fusarium* species of millet; *Fusarium verticillioides, F. scirpi, F. anthophilum* and *F. oxysporum*.

Materials and Methods

Source of millet varieties and plant extracts

This study was carried out at the University of Ibadan, Nigeria, from February to May, 2012. The seeds of Pear millet *(Pennisetum glaucum)* were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan Oyo state, Nigeria. The extracts of *Mangifera indica* var. julie (leaf methanol extraction) and *Jatropha curcas* (root back methanol extraction) were obtained from the Organic unit of the Chemistry Department, University of Ibadan, Nigeria. The leaves of *Moringa oleifera* and *Senna alata* were plucked from the garden of Botany Department, University of Ibadan, while the cassava (*Manihot esculenta*) peels were obtained from the cassava processing factory in the University neighbourhood.

Isolation and identification of *Fusarium* species

Soil samples were collected from various cereal growing farm sites across the five ecological zones in Southwestern Nigeria. Isolation of *Fusarium* species from the soil samples was carried out by soil plate method according to the procedure of Warcup (1950) Peptone Pentachloro-nitrobenzene Agar (PPA) medium. The isolated species were grown on Spezieller Nährstoffarmer Agar (SNA) medium for identification purpose. The identification and taxonomic classification of the isolated *Fusarium* spp. was aided by *Fusarium* identification manuals described by Leslie and Summerell (2006) and Fuskey *Fusarium* Interactive key reported by Seifert (1996).

Inoculums quantification and multiplication: The identified *Fusarium* species were quantified. The mycelial growth of young cultures of each *Fusarium* species were harvested, the solution was sieved with double folded cheese cloth to allow the passage of fungal spores which was later counted using haematocytometer. The spore suspension was then readjusted to 3.1×10^5 spores/ ml in this study.

Biocontrol of the pathogenic *Fusarium* species

Akanmu *et al.* (2013a) previously reported *F. anthophilum, F. verticillioides, F. oxysporum* and *F. scirpi* as the pathogenic strains among the isolated soil borne *Fusarium* spp. evaluated on different varieties of millet seedlings. These species were subjected to biological control measures using the

extracts of *M. indica, J. curcas, M. oleifera, S.alata* and *M. esculenta*.

Preparation of plant extracts: The extracts of *M. indica* and *J. curcas* were dissolved in 20% methanol and diluted with 80% sterile distilled water. After this, both extracts were further diluted to give concentrations of 0.15, 0.30 and 0.45 mg/ml. Fresh leaves of *M. oleifera* and *S. alata* were plucked while cassava peels (*M. esculenta*) were collected and properly washed in sterile water. Each of these items were separately rinsed in 5% NaOCl solution and rinsed again in two changes of sterile distilled water; then they were spread out on a sterilised surface to air-dry. The air-dried leaves and the dried cassava peels were blended in a sterile electric blender (Qlink blender, Model number: QBL-20L40, made in China). After this, the blended leaves and the cassava peel powder were weighed separately as 5, 10 and 15g before dispensing into 100 ml of solvent to obtain concentrations of 0.05, 0.01 and 0.15 g/ml, respectively.

***In-vitro* control of the pathogenic *Fusarium* species:**
Inhibitory potentials of plant extracts (*M. indica, J. curcas, M. oleifera, M. esculenta* (peels) and *S. alata*) were evaluated *in-vitro* on the pathogenic *Fusarium anthophilum, F. verticillioides, F. oxysporum* and *F. scirpi*. The experiment was carried out at three replicated concentration levels for each isolate. The periphery of eight (8) days old cultures of the pathogenic *Fusarium* spp. plated on PDA were picked with 5mm diameter cork borer and inoculated at the centre of each plate containing 9ml of PDA to 1ml of extract (Ramezani *et al.* 2002). The cultures and the control experiments were incubated at room temperature. The mycelia radial growth was consistently recorded at two (2) days interval for six (6) days. The percentage mycelia inhibition was calculated according to (Singh and Tripathi, 1999).

% inhibition = dc – dt/dc x 100

Where dc = Average increase in mycelial growth in control, dt = Average increase in mycelial growth in treatment.

***In-vivo* control of pathogenic *Fusarium* species**
Seed sterilization: The viable millet seeds were treated with 5% Sodium hypochlorite solution for three minutes, rinsed in two exchanges of sterile distilled water and air dried in laminar flow for 2 hours.

Screenhouse experiment: Excavated top soil from the farm area of Botany Department, University of Ibadan was sterilized using electric soil sterilizer. Three millet seeds per variety were planted per pot containing sterilized soil in a Completely Randomized Design (CRD) pot experiment and inoculated with 10ml of quantified ($3.1x\ 10^5$ spores/ml) spores of each *Fusarium* pathogen for 24 hours prior to planting. This was followed by inoculation with 10ml of plant extracts at concentration levels of 0.15, 0.30 and 0.45 mg/ml. The control experiments were positive (plant extracts and millet), neutral (mill*et al*one) and negative (pathogen and millet). Adequate management practices such as thinning, wetting and weeding were carried out.

Disease Assessment, data collection and analysis
Data on plant height (cm), stem girth (mm^2), leaf number and leaf area (cm^2) were collected at day 7 and 14.

Disease incidence rating: The percentage incidence of infected millet seedlings was estimated according to the method described by Michel *et al*. (1997):

% Disease incidence = n / N x 100

Where n = number of plant showing diseased symptoms with at least one leaf diseased, and N = Total number of sample used.

Scoring of Disease Severity: The disease severity was scored according to the method of Soonthornpoct *et al.* (2000).

0 = apparently healthy root or mesocotyl or crown tissue
1 = < 25% of tissue with disease rot symptoms
2 = 25–49% of tissue rotted
3 = 50–74%, of the tissue rotted
4 = 75% or greater of the roots rotted
5 = wilted or dead seedlings / completely rotted mesocotyl or crown tissue

Statistical Analysis: The data were subjected to Analysis of variance with Minitab version 15 (2011) statistical software, while the means were separated by Least Significant Difference (LSD) at 95% confidence level.

Results

The *Fusarium* spp. expressed highly significant ($p<0.001$) pathogenic effect on the millet seedlings as shown on the disease incidence, severity and reduced growth parameters recorded in table 1. The interactive effects of the extracts; *Mangifera indica* and *Jatropha curcas* thereafter produced highly significant control on the *Fusarium* spp. both *in vitro* and *in vivo* studies. Although, there were highly significant inhibitory effect of different concentrations of the extract on growths of mycelia *in vitro*, but not significant *in vivo* except on the leaf area. Also, highly significant effect was shown for time of observation both *in vitro* and *in vivo*.

In the first order of interactions; Extracts x *Fusarium* spp. (A x B) produced mycelia growth and increased in growth of most of the agronomic characters of millet with reduction in disease incidence and severity. On the other hand, Extracts x Time (A x D) and Concentration x Time (C x D) showed significant increase on the growth parameters, but reduced the effect of disease incidence and severity.

Table 1: Interactive effect of *M. indica* and *J. curcas* extracts at different concentrations with time in the control of pathogenic *Fusarium* spp. of millet seedlings.

	***In vitro* treatments**		***In-vivo* treatments**						
Source	**Df**	**Mycelia Growth**	**Df**	**Number of leaves**	**Plant height**	**Stem girth**	**Leaf Area**	**Disease incidence**	**Disease severity**
Extracts (A)	1	4.728**	2	2.688**	1064.277**	13.466^{ns}	266.223**	1.623**	105.250**
***Fusarium* spp. (B)**	3	6.487**	5	1.299*	47.908**	12.366^{ns}	28.006*	0.097**	8.881**
Concentration (C)	3	6.000**	2	0.827^{ns}	13.183^{ns}	11.275^{ns}	187.155**	0.011^{ns}	0.148^{ns}
Time (D)	2	351.48**	1	82.003**	6867.437**	2.721^{ns}	9268.778**	0.008^{ns}	0.309^{ns}
A x B	3	0.298**	10	1.066*	122.225**	8.642^{ns}	38.234*	0.105**	8.698**
A x C	3	5.291**	4	0.438^{ns}	15.183^{ns}	10.258^{ns}	121.369**	0.013^{ns}	0.231^{ns}
A x D	2	0.234**	2	4.596**	330.015**	8.612^{ns}	166.256**	0.002^{ns}	1.170^{ns}
B x C	9	0.566**	10	0.249^{ns}	29.233^{ns}	9.844^{ns}	20.307^{ns}	0.005^{ns}	0.119^{ns}
B x D	6	0.840**	5	1.003*	18.895^{ns}	9.56^{ns}	18.766^{ns}	0.004^{ns}	0.894^{ns}
C x D	6	0.088**	2	134.827**	11235.247**	6.802^{ns}	156.403**	0.004^{ns}	0.12^{ns}
A x B x C	9	0.323**	20	0.227^{ns}	29.069^{ns}	9.645^{ns}	14.621^{ns}	0.006^{ns}	0.152^{ns}
A x B x D	6	0.063**	10	0.618^{ns}	23.726^{ns}	10.401^{ns}	20.595^{ns}	0.003^{ns}	1.055*
A x C x D	6	0.243**	4	12.086**	1163.437**	8.307^{ns}	130.055**	0.001^{ns}	0.12^{ns}
B x C x D	18	0.289**	10	1.138**	48.925*	8.839^{ns}	12.441^{ns}	0.004^{ns}	0.042^{ns}
A x B x C x D	18	0.226**	20	1.02**	83.970**	9.734^{ns}	8.680^{ns}	0.002^{ns}	0.025^{ns}
Error		1.727		72	4565.688	2094.46	2641.878	2.846	104
Total		7094.43		5219	171567.1	3472.719	37374.13	12.33	666
Corrected Total		788.787		557.145	46269.24	3126.242	16084.07	8.029	470

Note: Highly significant ($p<0.001$) = **, Significant ($p<0.05$) = *, ns = not significant

The second order of interactions for the treatments of Extracts x Concentration x Time (A x C x D) also produced similar significant effect for the growth characters of millet, while disease incidence and severity were not significant. The third order of interaction; Extracts x *Fusarium* spp. x Concentration x Time was highly significant *in vitro*, but showed significant increase on the number of leaves and plant height *in vivo* (Table 1).

Similarly, the effect of the extracts of *Moringa oleifera*, *Manihot esculenta* and *Senna alata* was observed (Table 2). The Extracts, Concentration and Time were highly significant ($p<0.001$), while plants treated with *Fusarium* spp. produced high growth of mycelia with highly significant disease incidence and severity which reduced most of the growth parameters of millets. The first order of interactions; Extracts x *Fusarium* spp. (A x B), Extracts x Concentration (A x C) and Concentration x Time (C x D) were highly significant ($p<0.001$) for most characters evaluated *in vitro* and *in vivo*. The Extracts x Concentration levels x Time (A x C x D) level of interaction was not significant ($p<0.05$) *in vitro*, but highly significant for growth characters *in vivo* and produced reduction effects for disease incidence and severity. Whereas, non significant effect was shown for the interactions the order of interaction; Extracts x *Fusarium* spp. x Concentration levels x Time (A x B x C x D) (Table 2).

Table 2: Interactive effect of *M. oleifera*, *M. esculenta* and *S. alata* extracts at different concentrations with time in the control of pathogenic *Fusarium* spp. of millet seedlings.

	In vitro Treatments		*In vivo* Treatments						
Source	**Df**	**Mycelia growth**	**Df**	**Number of leaves**	**Plant height**	**Stem girth**	**Leaf Area**	**Disease incidence**	**Disease severity**
Extracts (A)	2	0.251**	3	1.817*	252.473**	1.720**	300.819**	0.684**	57.040**
***Fusarium* spp. (B)**	3	1.698**	5	1.754*	56.960*	0.188*	79.587*	0.262**	15.698**
Concentration (C)	3	2.295**	2	16.924**	2682.032**	0.174*	207.640**	0.106*	1.370^{ns}
Time (D)	2	77.478**	1	479.225**	25796.87**	4.661**	15157.91**	0.036^{ns}	6.750*
A x B	6	0.159**	15	0.947*	51.086**	0.158**	36.572*	0.056**	4.944**
A x C	6	0.248**	6	5.331**	314.604**	0.372**	72.846**	0.016^{ns}	0.522^{ns}
A x D	4	0.015^{ns}	3	2.755**	3.708^{ns}	0.362**	169.512**	0.007^{ns}	2.386*
B x C	9	0.143**	10	1.132*	135.015**	0.47^{ns}	29.827^{ns}	0.021^{ns}	0.509^{ns}
B x D	6	0.452**	5	1.680*	5.666^{ns}	0.064^{ns}	27.966^{ns}	0.01^{ns}	1.006^{ns}
C x D	6	0.086**	2	11.877**	2179.615**	1.853**	153.426**	0.021^{ns}	0.333^{ns}
A x B x C	18	0.094**	30	0.536^{ns}	26.056^{ns}	0.041^{ns}	15.206^{ns}	0.025^{ns}	0.209^{ns}
A x B x D	12	0.024^{ns}	15	0.611^{ns}	18.361^{ns}	0.044^{ns}	21.986^{ns}	0.004^{ns}	0.797^{ns}
A x C x D	12	0.012^{ns}	6	3.501**	246.392**	0.599**	51.717*	0.011^{ns}	0.145^{ns}
B x C x D	18	0.048**	10	0.491^{ns}	101.420**	0.097*	11.701^{ns}	0.006^{ns}	0.720^{ns}
A x B x C x D	36	0.01^{ns}	30	0.215^{ns}	14.343^{ns}	0.039^{ns}	8.793^{ns}	0.009^{ns}	0.840^{ns}
Error		4.426		116.667	5137.73	15.009	5027.58	5.464	184
Total		3020.106		6325	178854	395.85	56168.6	20.295	864
Corrected Total		182.214		799.479	49723.6	43.905	25617.4	11.557	560.657

Note: Highly significant ($p<0.001$) = **, Significant ($p<0.05$) = *, ns = not significant

The result from table 3 shows that *M. indica* (4.55cm) produced higher inhibitory effect on the mycelia growth of *Fusarium* spp. than *J. curcas* (4.81cm), both expressed significant ($p<0.05$) effects compared with positive, neutral and negative controls. In the *in vivo* experiment, *J. curcas* showed better performances, and significantly enhanced the number of leaves, plant height, stem girth and the leaf area of the pathogen treated millet seedlings. The Positive control only recorded the most significant performance on the growth of millet seedlings compared to the neutral control. Similarly, *in vitro* evaluation showed that the aqueous extracts of *M. oleifera, M. esculenta* and *S. alata* produced inhibitory effect on mycelia growth of *Fusarium* spp., *M. esculenta* (2.61cm) showed the most significant ($p<0.05$) antagonistic effects, and closely followed by *M. oliefera* (2.55 cm) and *S. alata* (2.53cm).

Table 3: Antagonistic effect of plant extracts on the pathogenic *Fusarium* spp. of millet seedlings.

		In- vitro	*In vivo* treatments					
	Extracts	**Mycelia growth(cm)**	**Number of leaves**	**Plant height(cm)**	**Stem girths(cm)**	**Leaf Area(cm²)**	**Disease incidence**	**Disease severity**
Extracts A	***M. indica***	4.55c	3.89b	21.32b	0.91d	8.04c	0.05a	0.25b
	J. curcas	4.61b	3.88b	21.63a	1.43b	9.71a	0.04b	0.17c
	Positive control	Na	3.94a	20.67c	1.99a	9.16b	0.04c	0.17c
	Neutral control	4.86a	3.83c	18.73d	1.00c	7.94d	0.11d	0.48a
	Std. Error	0.08	0.56	0.420	0.30	0.34	0.11	0.67
Extracts B	***M. oleifera***	2.55c	3.76a	18.80a	0.93d	9.62a	0.11b	0.44c
	M. esculenta	2.61b	3.54c	17.42b	0.96c	8.83d	0.07d	0.37d
	Senna alata	2.53c	3.45d	17.77c	1.00a	9.23c	0.13a	0.62a
	Positive control	Na	3.68b	17.23c	0.97b	9.37b	0.04c	0.17e
	Neutral control	2.68a	3.68b	16.12d	0.87e	6.61e	0.11b	0.46b
	Std. Error	0.10	0.06	0.41	0.02	0.40	0.01	0.77

Means with the same alphabet across the column are not significantly ($p<0.05$) different from one another.

Table 4: Effectiveness of extract concentration in the control of *Fusarium* spp. of Millet seedlings

		In vitro	*In vivo* treatment					
	Concentrations	**Mycelia Growth(cm)**	**Number of leaves**	**Plant height(cm)**	**Stem girths(cm)**	**Leaf Area(cm²)**	**Disease incidence**	**Disease severity**
Extracts A	**0.15 mg/ml**	4.82b	3.69d	19.86b	0.84d	6.59d	0.12b	0.82a
	0.30 mg/ml	4.78c	3.82c	19.88a	1.41a	8.90a	0.12a	0.78b
	0.45 mg/ml	4.25d	3.86a	19.26c	0.86c	8.84b	0.10c	0.74c
	Control	4.86a	3.83b	18.73d	1.00b	7.94c	0.11b	0.48d
	Std. error	0.11	0.56	0.44	0.30	0.34	0.01	0.67
Extracts B	**0.05 g/ml**	2.67b	3.18c	12.31d	0.87c	7.36c	0.17a	0.93a
	0.10 g/ml	2.54c	3.78a	19.55b	0.93a	8.15b	0.15b	0.85b
	0.15 g/ml	2.37d	3.77a	20.00a	0.91b	9.73a	0.11c	0.74c
	Control	2.68a	3.68b	16.12c	0.87c	6.61d	0.11c	0.46d
	Std. Error	0.12	0.05	0.35	0.02	0.35	0.01	0.07

Means with the same alphabet across the column are not significantly ($p<0.05$) different from one another.

All the extracts expressed significant mycelia inhibition when compared to the neutral control (2.68cm). *M. oleifera* extract had the most effective antagonistic activity *in vivo* with the highest performances in the number of leaves, plant height and leaf area, followed by *M. esculenta* and then *S. alata*. The positive control supported the growth best (Table 3).

The efficacy of plant extracts against the pathogenic *Fusarium* spp. was enhanced as the concentration of the extracts increases as observed *in vitro*. The *in vivo* experiments involving the extracts of *M. indica* and *J. curcas* produced significant ($p<0.05$) growth on plant height, stem girth and leaf area at 0.30 mg/ml concentration whereas, disease incidence and severity were effectively antagonized at 0.45 mg/ml. Also, for *M. oleifera*, *M. esculenta* and *S. alata* applied at 0.05, 0.10, and 0.15 g/ml concentrations, the *in vitro* and *in vivo* were mostly controlled significantly at 0.15 g/ml, while the disease incidence and severity were significantly ($p<0.05$) antagonized at this concentration, followed by 0.10 g/ml and 0.05 g/ml concentrations respectively (Table 4).

The susceptibility of the pathogenic *Fusarium* spp. to *M. indica* and *J. curcas* extracts was in order of *F. oxysporum, F. scirpi, F. anthophilum* and *F. verticillioides in vitro*. Also, the extracts of *M. oleifera*, *M. esculenta* and *S. alata* controlled *F. oxysporum, F. scirpi*, *F. verticillioides* and *F. anthophilum* in the order. In the *in vivo*, the pathogenic effect of *F. scirpi* was significant and mostly suppressed by the plant extracts, followed by *F. oxysporum*, *F. verticillioides* and *F. anthophilum* as evaluated from the growth parameters and disease severity (Table 5).

The effect of time measured based on the days of data collection produced significant ($p<0.05$) effect on the mycelia growth of *Fusarium* spp. *in vitro*. This result was affirmed *in vivo*, for day 14 recorded when significant effect was observed on the growth parameters with lowered disease incidence and severity (Table 6).

Table 5: Susceptibility of *Fusarium* spp. to the plant extracts treatments.

		In vitro	*In vivo* treatments					
	Fusarium spp.	**Mycelia Growth (cm)**	**Number of leaves**	**Plant height (cm)**	**Stem girths (cm)**	**Leaf Area (cm^2)**	**Disease incidence**	**Disease severity**
Extracts A	***F. anthophilum***	4.64c	3.50e	18.33e	0.74f	7.11d	0.16b	1.33b
	F. verticillioides	4.27d	3.77c	19.82c	0.78d	7.86b	0.14c	0.82e
	F. oxysporum	4.91a	3.82b	19.94b	0.82c	7.86b	0.13d	0.96c
	F. scirpi	4.90a	3.89a	20.50a	0.88b	8.69a	0.12e	0.91d
	Negative control	Na	3.61d	16.05f	0.76e	6.57e	0.26a	1.92a
	Neutral control	4.86b	3.83b	18.73d	1.00a	7.94c	0.11f	0.48f
	Std. error	0.11	0.79	0.63	0.42	0.48	0.02	0.94
Extracts B	***F. anthophilum***	2.490e	3.29e	16.40d	0.86d	8.05d	0.22b	1.50b
	F. verticillioides	2.438d	3.54d	17.59c	0.88b	8.86b	0.17c	1.01c
	F. oxysporum	2.719a	3.56c	18.03b	0.87c	8.17c	0.15e	0.90e
	F. scirpi	2.605c	3.71a	18.36a	0.96a	9.39a	0.17d	0.99d
	Negative control	Na	3.56c	15.17f	0.72e	5.92f	0.26a	1.92a
	Neutral control	2.682b	3.68b	16.12e	0.87d	6.61e	0.11f	0.46f
	Std. Error	0.120	0.08	0.50	0.03	0.49	0.02	0.09

Means with the same alphabet across the column are not significantly ($p<0.05$) different from one another.

Table 6: The effect of time on the *in vitro* and *in vivo* control of pathogenic *Fusarium* spp. of millet seedlings.

	***in vitro* treatments**		***in vivo* treatments**						
	Time	**Mycelia Growth (cm)**	**Time**	**Number of leaves**	**Plant height (cm)**	**Stem girths (cm)**	**Leaf Area (cm^2)**	**Disease incidence**	**Disease severity**
Extracts A	**Day 2**	2.72c	**Day 7**	3.29b	15.06b	1.13a	2.76b	0.12a	0.81a
	Day 4	4.77b	**Day 14**	4.30a	24.27a	0.94b	13.46a	0.11b	0.75b
	Day 6	6.54a	Na	-	-	-	-	-	-
	Std. Error	0.10	Na	0.45	0.36	0.25	0.28	0.01	0.06
Extracts B	**Day 2**	1.79c	**Day 7**	2.52b	9.56b	0.80b	2.49b	0.15a	0.96a
	Day 4	2.65b	**Day 14**	4.63a	25.02a	1.01a	14.33a	0.13b	0.71b
	Day 6	3.25a	Na	-	-	-	-	-	-
	Std. Error	0.10	Na	0.04	0.29	0.02	0.28	0.09	0.05

Means with the same alphabet across the column are not significantly ($p<0.05$) different from one another.

Discussion

In the recent times, the research across the globe has focused on harnessing the potentials of natural chemicals and their uses in integrated plant protection (Kiran *et al*. 2006). This was evident in researches conducted on plant disease control using plant extracts as alternative measures to replace the conventional synthetic pesticides (Raghuwanshi *et al*. 2003; Agbenin and Marley 2006; Viudamartos *et al*. 2007) due to its antifungal, antioxidant and photochemical potentials (Jonathan *et al*. 2012). Thus, the suitable condition for the optimum performances of the plant extracts in suppressing pathogenic *Fusarium* spp. of millet seedlings was investigated in this study.

The evaluation of *in vitro* and *in vivo* interactive effects of the plant extracts at different concentration levels against *Fusarium* spp. was observed over a period of time. Except in the co-interactions of the *Extracts x Time* at all levels of interactions in *M. oleifera, M. esculenta* and *S. alata* extracts effectively inhibited the mycelia growths *in-vitro.* This observation was in accordance with several reports on inhibitory effects of some botanicals against *Fusarium* spp. (Agbenin and Marley 2006; Babu *et al*. 2008). The interaction of the four *Fusarium* spp. recorded high disease incidence and severity on the millet seedlings *in vivo*, and could be attributed to the virulence of one or more of the *Fusarium* spp reported (Reid *et al*. 1999; Caesar 2003; Antônia *et al*. 2006; Noëlani *et al*. 2007; Anjali *et al*. 2012; Akanmu *et al*. 2013a).

The pathogenic effects of *Fusarium* spp. were effectively suppressed by the interactions of the plant extracts, indicating the efficacy and the phytofungicidal potentials of the plant extracts against the pathogens as earlier reported by Caesar (2003) and Babu *et al*. (2008). As expected, no significant disease severity was recorded for plant extracts applied at different concentration levels as well as the time of observation. This result which reflects in interactions of these parameters could be due to the application of the plant extracts at non toxic concentration to plants' health as affirmed by Akanmu *et al*. (2013b). Moreso, the non significance of the *Time of observation* in disease expression could possibly indicate the efficacy and prompt activities of the combined extracts in suppressing the pathogenic potentials of the *Fusarium* spp., thus, establishing the potency of some plant extracts reported by Paola *et al*. (2010). The interactions of the treatments on the growth parameters showed highly significant ($p<0.001$) effects for; *Extract x Fusarium* spp., *Extract x Time, Concentration levels x Time* and also *Extract x Concentration levels x Time* as similarly

observed by Olawuyi *et al.* (2013a). The efficacy of the treatments has also been reported to show synergistic activity on the extracts of clove, guava and lemon grass with antimicrobial drugs as reported by Zafar *et al*., (2010). However, no significant interactive effects was expressed in the treatments; *Fusarium* spp. *x Time, Extracts x Fusarium* spp. *x Concentration levels* and *Extracts x Fusarium* spp. *x Time* as similarly reported by Olawuyi *et al*. (2013b). The plant extracts; *J. curcas* and *M. indica* at 0.45mg/ml, also *M. oleifera, M. esculenta* and *S. alata* at 0.15g/ml concentrations appeared the most effective treatments of *Fusarium* scirpi, *F. oxysporum*, *F. verticillioides* and *F. anthophilum* in the order of arrangement. However, in natural environment, the treatment; *Extract x Concentration levels x Time* suggest that the interactive treatments of the extracts; *J. curcas* and *M. indica* at 0.45mg/ml, as well as *M. oleifera, M. esculenta* and *S. alata* at 0.15g/ml concentrations as modulated environment that could effectively suppressed the sole or combined effects of *Fusarium* spp. and enhances plant growth when observed over the period of Time. This interactive control measure was also supported by Pal and Gardner (2006) who considered biological control in plant as the net positive result arising from a variety of specific and non-specific interactions.

Therefore, the study of the interactions of the botanicals in management of plant pathogen is another approach that should be integrated in plant disease control. This provides valuable information on the interactive effects of treatment factors that best enhances the optimum performance of the botanicals against the pathogenic *Fusarium* species of millet seedlings in this study.

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