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IN VITRO SCREENING OF SELECTED HERBICIDES ON RHIZOSPHERE MYCOFLORA FROM YELLOW PEPPER (*CAPSICUM ANNUM L* VAR. NSUKKA YELLOW) SEEDLINGS IN NSUKKA, ENUGU STATE, NIGERIA

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ABSTRACT

In vitro screening of five selected herbicides at different concentrations on rhizosphere mycoflora from yellow pepper (*capsicum annum* L var. Nsukka yellow) seedlings at Nsukka were investigated. The herbicides employed for this study were Paraquat, Glyphosate, Primextra, Atrazine and Linuron. The isolated rhizosphere mycoflora organisms screened were *Rhizopus stolonifer (*Ehren ex. Fr*)* Lind, *Trichoderma harmatum* Bain aggr; *Aspergillus niger* Van Tiegh; *Sclerotium rolfsii* Sacc; *Penicillium* sp; *Alternaria* sp; *Fusarium solani (*Mart*)* Sacc and *Rhizoctonia* sp. The results showed that all the herbicides at different concentrations of 0.5 µg/ml a.i, 1.0 µg/ml a.i, 2.0 µg/ml a.i, 5.0 µg/ml a.i and 10.0 µg/ml a.i inhibited the mycelial growth of the isolated rhizosphere fungi. Growth inhibition of the rhizosphere fungi increased with increasing concentrations of each herbicide. Paraquat, Glyphosate and Atrazine showed higher inhibition potency on all the fungal mycelial growth than Primextra and Linuron. The significance of these results is discussed.

KEYWORDS*: In vitro* screening, herbicides, Rhizosphere mycoflora, yellow pepper seedlings.

INTRODUCTION

Weeds constitute one of the biggest problems in agriculture that not only do they reduce the yield and quality of crops but also utilize essential nutrients meant for the crop. Hence, weed control is essential for increase in agricultural production. Herbicides are one of the major groups of pesticides which include substances or cultured biological organisms used to kill or suppress the growth of weeds which are unwanted plants in order to minimize the cultivation costs as well as to sustain high yield (Cork and Krueger, 1992). They are the major group of pesticide which contribute to the increased and economically production of crop plants and minimize human toil in agricultural production (Subhani et.al; 2000). They are widely used for the control of weeds in modern agricultural practices in Nigeria and many other countries all over the world.

However, when applied to the field crops, herbicides not only control targeted weeds but may also have potential impact on microfloral populations in the soil (Zabaloy et al; 2008, Ayansina and Oso, 2006). The manner in which rhizosphere microflora respond to the application of herbicides when introduced to their environment depends on the type of microfloral organisms involved, the concentration and application rates and environmental factors (Sabiomo et.al. 2011). The sustainable agriculture involves optimizing agricultural resources and the same time maintaining natural resources. In achieving this optimization, the soil microbial community composition is a great importance because they play a crucial role in carbon flow, nutrient recycling, nitrogen cycle, organic matter decomposition which affect soil fertility and plant growth and hence occupy a unique position in biological cycles in terrestrial habitat (Hutsch, 2001; Chauhan et.al; 2006, Tripathi et al; 2006, Pandey et al, 2007). The soil microbial biomas is considered as active nutrient pool to plants and plays a vital role in nutrient cycling and decomposition in ecosystem. Gupta et.al; (2011) indicated that herbicides had significant deleterious effect on soil fungi, micorrhizal spore numbers and percentage root colonization and this have increased injuries to the beneficial soil microbial organisms.

The unique environment immediately surrounding the roots is called the rhizosphere. It is the region around roots where simple sugars, amino acides and many other compounds are executed by plants and made available to the microorganisms (Campbell, 1989). The rhizosphere region is biologically more active than the soil farther away (Shalaby et.al; 2002). The increased activity in this region is believed to be due to the presence of greater microbial populations, which in turn, is influenced by the various biologically active chemicals exuded by the plant roots.

The effect of herbicides on the rhizosphere mycofloral organisms on farms of yellow pepper (*C annum*) is likely to involve both direct and indirect effects. The direct effect involves reduction in rhizosphere exudates as earlier stated by Gupta et.al. (2011).

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Ayansina and Oso, (2006) reported that herbicide Antrazine and Metolachlor treatments at both the recommended and above recommended rates resulted in decreased microbial counts.

The assessment of unforeseen consequences due to use of herbicides is important in providing deeper insight for herbicide risk management as stated by Zain et.al. (2013). Rennie et.al; (1985) and Brazauskiene (1998) reported that herbicides might be stimulatory, inhibitory or toxic to specific groups of microorganisms. This study was therefore undertaken to assess the effect of different concentrations of five commonly used herbicides on some isolated rhizosphere fungi on yellow pepper (*C.annum*) seedlings at Nsukka.

MATERIALS AND METHOD

Study Site and Soil Properties.

This study was carried out at Ikwoka village of Obimo in Nsukka Local Government Area of Enugu State of Nigeria. The study period covered March to May 2014. The study was carried out on yellow pepper *(Capsicum annum L.* var. Nsukka yellow*)* planted in January/February, 2014.

Physical and chemical analysis of rhizosphere soil sample on this yellow pepper seedlings was conducted in Soil Science Departmental Laboratory of University of Nigeria, Nsukka, Nigeria. The analysis carried out covered soil texture, moisture content, organic matter content and pH (Dongmo and Oyeyiola, 2006; Oyeyiola, 2009, and Eze and Amadi, 2014).

Isolation of Rhizosphere Test fungi.

The methods of Eze and Amadi, (2014) Mansour and Hamdi, (1983), were employed. Six weeks old yellow pepper seedlings were carefully uprooted from the farm and carried in a sterilized polyethylene bags to the laboratory. The roots were manually shaken to remove loose soil particles. The roots were cut into 2 mm segments and 10g representative samples shaken into 90ml of sterile distilled water. Serial dilutions were made from this stock and plated onto Potato Dextrose Agar (PDA) for the isolation of rhizosphere fungi. Inoculated plates were incubated at laboratory temperatures of 28-30^0C± 20C. Counts were taken after 3 and 4 days. Pure cultures of isolated fungi were obtained through several transfers. Pure isolates were identified using standard mycological methods and were preserved in PDA slants at 28-30^0C as stock cultures for *In vitro* herbicide studies.

In Vitro herbicide inhibition of isolated *Rhizosphere Fungi.*

The herbiscides used for this study were obtained from local Agrochemical dealer in Odenigbo, Nsukka, Enugu State of Nigeria. Five herbicides were selected based on those most frequently used by farmers. The herbicides used were: Paraquat (1-1 dimethyl -4-bipyridylium dichloride), Glyphosate (N (phosphono methyl) glycine-chemical family organo phosphorus Primextra (a combination of Atrazine and Metolachlor, Atrazine, Atrylone 80 wp, trade mark of Insis Ltd, a product of syngenta and Linuron (N-3, 4- dichlorophenyl) – N – methoxy – N – Methyl urea). The herbicides were individually , incorporated into PDA sterilized at 121^0C for 15 min after cooling to 37^0C – 40^0C at concentrations of 0.5, 1.0, 2.0, 5.0 and 10.0 µ*g*/ml active ingredient (a.i) while the control was PDA incorporated with 1ml of distilled water. About 20ml of herbicide – PDA medium was poured into 90mm sterilized Petri dishes of 9cm diameter and allowed to solidify before inoculations. Fungal subcultures of 6day old were transferred aseptically using sterile innoculation needle to the centre of the herbicide - PDA medium and control plates. The plates were then covered and sealed followed by incubation at 28^0C – 30^0C in darkness.

The effect of herbicides on the fungal species was measured by the radial growth of fungal colony in both control and herbicide – PDA plates for seven consecutive days using millimeter ruler. The measurements were expressed as inhibition percentage of the colony calculated using the formula of Pandey et.al; (1982).

$$\text{Percentage Growth Inhibition} = \frac{DC - DT}{DC} \times \frac{100}{1}$$

Where DC is the average diameter of fungal colony in control and DT is the average diameter of fungal colony with herbicide treatments.

Statistical Analysis.

The experiments were conducted using Complete Randomized Design (CRD) with three replications. The mean percentage *In Vitro* inhibition of the fungal growth by herbicides was obtained and data analysed using One Way Analysis of Variance (ANOVA).

RESULTS

The results of soil analysis showed that the pH was 6.5 indicating slight acidity (Table 1). The texture of the soil was found to be sandy-loam and the organic matters content was quite high (6.9%). The results also show that the isolated rhizosphere fungi were *Rhizopus* stolonifer (Ehren ex Fr.) Lind, *Trichoderma harmatum (*Ben*.)* Bain aggr, *Aspergillus niger* VanTiegh, Sclerotium *rolfsii* Sacc; Penicillium sp. Alternaria sp; *Fusarium solani (*Mart*)* Sacc; and Rhizoctonia sp. These rhizosphere fungal isolates reacted differently to the five herbicides and at their different concentrations. The individual effects of the herbicides on the different fungal isolates are presented in Tables 2- 6. All the different concentrations of the herbicides screened were inhibitory on the mycelial growth of the rhizosphere fungal isolates. Growth inhibition became more severe with increasing concentration of the herbicides though the percentage inhibition varied from one fungus to another and also from one herbicide to another. Statistial analysis showed that the herbicides had significant effect (P≤ 0.05) on the mycelia growth of the isolated rhizosphere fungi. Paraguat, Glyphosate, Atrazine and Linuron showed higher potency of inhibition on the mycelia growth than Primextra. At 1.0 µ*g*/ml a.i and above concentrations, almost all herbicides showed more than 50% inhibition on the mycelia growth in all isolated rhizosphere fungi screened. Fusarium solani was most sensitive to all the fungicides tested at all the different concentrations.

Table 1: Characteristic Properties of Rhizosphere soil Employed for the study.

Soil characteristics	Values
pH	6.7
Moisture Content	5.4
Water Holding Capacity (m/g)	0.5
Organic Matter Content (%)	6.9
Sand	76.5%
Silt	18.2%
Clay	5.3%
Soil texture	sandy clay

Table 2: *In Vitro* Effect of Paraquat - PDA media on Mycelial Growth of Rhizosphere soil fungi of yellow pepper (*Capsicum* annum) seedlings.

Rhizosphere soil fungi	**Concentrations of herbicide in µg/ml a.i**											
	0.5 Mycelia Growth (mm)	% inhibition	1.0 Mycelial Growth (mm)	% inhibition	2.0 Mycelial growth (mm)	% inhibition	5.0 Mycelial growth (mm)	% inhibition	10.0 Mycelial Growth (mm)	% inhibition	Control Mycelial growth (mm)	% inhibition
Rhizopus stolonifer	4.87	40.10	3.76	53.75	2.68	67.04	1.32	83.76	0.37	95.45	8.13	-
Trichoderna harmatum	5.33	35.16	3.68	55.23	2.67	67.15	1.63	80.17	0.47	94.28	8.22	-
Aspergillus niger	4.21	48.22	3.34	61.07	1.28	84.26	0.86	89.96	0.00	100	8.58	-
Sclerotium rolfsii	4.77	36.90	3.32	56.08	2.62	65.34	1.38	81.75	0.13	98.28	7.56	-
Penicillium sp	4.58	28.66	3.55	44.70	2.64	58.88	0.00	100	0.00	100	6.42	-
Alternaria sp	4.81	35.61	3.68	50.74	2.17	70.95	1.14	84.74	0.00	100	7.47	-
Fusarium solani	3.74	51.36	2.27	70.48	1.25	83.75	0.15	98.05	0.00	100	7.69	-
Rhizoctonia sp	3.73	45.39	2.64	61.35	1.48	78.33	0.36	94.73	0.00	100	6.83	-

L S D (P≤0.05) 0. 83

Table 3: *In Vitro* Effect of Glyphosate-PDA media on Mycelial Growth of Rhizosphere soil fungi of yellow pepper *Capsicum* (annum) seedlings.

Rhizosphere soil fungi	**Concentrations of herbicide in µg/ml a.i**											
	0.5 Mycelia Growth (mm)	% inhibition	1.0 Mycelial Growth (mm)	% inhibition	2.0 Mycelial growth (mm)	% inhibition	5.0 Mycelial growth (mm)	% inhibition	10.0 Mycelial Growth (mm)	% inhibition	Control Mycelial growth (mm)	% inhibition
Rhizopus stolonifer	4.47	41.80	3.68	52.08	2.34	69.53	1.13	85.29	0.00	100	7.68	-
Trichoderna harmatum	4.14	43.13	3.55	51.24	2.49	65.80	1.25	82.83	0.26	96.43	7.28	-
Aspergillus niger	4.24	43.16	3.34	55.23	2.42	67.56	1.00	86.60	0.00	100	7.46	-
Sclerotium rolfsii	4.12	53.45	3.25	63.28	2.22	74.91	1.31	84.63	0.12	98.64	8.85	-
Penicillium sp	4.16	49.51	3.67	55.46	2.94	64.32	1.13	86.29	0.22	97.33	8.24	-
Alternaria sp	4.14	45.09	3.23	57.16	1.87	75.20	0.16	97.90	0.00	100	7.54	-
Fusarium solani	3.89	52.44	2.02	75.31	1.11	86.43	0.00	100	0.00	100	8.18	-
Rhizoctonia sp	3.24	56.97	2.43	67.73	1.14	84.86	0.47	93.76	0.00	100	7.53	-
L S D	**(P≤0.05) 0.57**											

Table 4: *In Vitro* Effect Primextra – PDA media on Mycelial Growth of Rhizosphere fungi of yellow pepper (*Capsicum* annum) seedlings.

Rhizosphere soil fungi	Concentrations of herbicide in µg/ml a.i											
	0.5 Mycelia Growth (mm)	% inhibition	1.0 Mycelial Growth (mm)	% inhibition	2.0 Mycelial growth (mm)	% inhibition	5.0 Mycelial growth (mm)	% inhibition	10.0 Mycelial Growth (mm)	% inhibition	Control Mycelial growth (mm)	% inhibition
Rhizopus stolonifer	5.75	23.64	4.88	35.19	3.86	43.02	2.48	67.06	1.28	83.00	7.53	-
Trichoderna harmatum	5.20	32.99	4.54	41.49	3.11	59.92	2.22	71.39	1.50	80.67	7.76	-
Aspergillus niger	5.47	33.62	4.28	48.06	3.00	63.59	1.85	77.55	0.87	88.79	8.24	-
Sclerotium rolfsii	4.78	36.10	3.37	54.95	2.66	64.44	1.54	79.41	0.58	92.25	7.48	-
Penicillium sp	4.67	40.36	3.56	54.53	2.32	70.37	1.44	81.61	0.96	87.74	7.83	-
Alternaria sp	5.53	33.77	4.55	45.51	3.62	56.65	2.32	72.22	1.57	81.20	8.35	-
Fusarium solani	4.28	45.48	2.77	64.47	1.35	82.80	0.73	90.70	0.00	100	7.85	-
Rhizoctonia sp	4.93	36.63	3.74	51.93	2.86	63.24	0.22	71.47	1.36	82.52	7.78	-
L S D	**(P≤0.05) 0.58**											

Table 5: *In Vitro* Effect of Atrazine –PDA media on Mycelial Growth of Rhizosphere soil fungi of yellow pepper (*Capsicum* annum) seedlings.

Rhizosphere soil fungi	**Concentrations of herbicide in µg/ml a.i**											
	0.5 Mycelia Growth (mm)	% inhibition	1.0 Mycelial Growth (mm)	% inhibition	2.0 Mycelial growth (mm)	% inhibition	5.0 Mycelial growth (mm)	% inhibition	10.0 Mycelial Growth (mm)	% inhibition	Control Mycelial growth (mm)	% inhibition
Rhizopus stolonifer	4.83	29.59	3.54	48.40	2.27	66.91	1.52	77.84	0.36	94.75	6.86	-
Trichoderna harmatum	4.14	49.33	3.62	55.69	2.18	73.32	1.49	81.76	0.68	91.68	8.17	-
Aspergillus niger	4.68	44.22	2.64	68.53	1.45	82.72	0.83	90.12	0.00	100	8.39	-
Sclerotium rolfsii	4.87	31.43	3.34	55.82	2.17	71.30	1.22	83.86	0.12	98.41	7.56	-
Penicillium sp	4.27	50.23	3.38	60.61	2.05	76.12	0.68	92.07	0.00	100	8.58	-
Alternaria sp	4.68	39.92	3.46	55.58	2.33	70.08	1.17	84.98	0.35	95.51	7.79	-
Fusarium solani	3.36	57.36	2.11	73.22	1.26	84.01	0.00	100	0.00	100	7.88	-
Rhizoctonia sp	3.23	54.70	2.64	62.97	1.27	82.19	0.00	100	0.00	100	7.13	-

LSD (P≤ 0.05) 0.64

Table 6: *In Vitro* Effect of Linuron-PDA media on mycelia Growth of Rhizosphere soil fungi of yellow pepper (*Capsicum* annum) seedlings.

Rhizosphere soil fungi	Concentrations of herbicide in µg/ml a.i											
	0.5 Mycelia Growth (mm)	% inhibition	1.0 Mycelial Growth (mm)	% inhibition	2.0 Mycelial growth (mm)	% inhibition	5.0 Mycelial growth (mm)	% inhibition	10.0 Mycelial Growth (mm)	% inhibition	Control Mycelial growth (mm)	% inhibition
Rhizopus stolonifer	5.23	30.64	4.38	41.91	2.17	71.22	1.62	78.51	0.44	94.16	7.54	-
Trichoderna harmatum	4.89	37.79	3.75	52.29	2.36	69.97	1.27	83.84	0.55	93.00	7.86	-
Aspergillus niger	5.18	36.91	4.33	47.26	2.95	64.10	1.35	83.56	0.82	90.01	8.21	-
Sclerotium rolfsii	5.15	38.10	4.42	46.88	2.66	68.03	1.18	85.82	0.34	95.91	8.32	-
Penicillium sp	4.63	41.10	3.84	51.15	2.28	70.99	0.95	87.91	0.00	100	7.86	-
Alternaria sp	4.78	38.48	3.37	56.63	2.00	74.26	0.82	89.45	0.00	100	7.77	-
Fusarium solani	3.76	51.98	2.24	77.39	0.88	88.76	0.00	100	0.00	100	7.83	-
Rhizoctonia sp	4.12	47.65	2.89	63.28	1.36	82.72	0.55	93.01	0.00	100	7.87	-

LSD (P≤ 0.05) = 0.86

DISCUSSION

The results of this study showed that different herbicides exhibited high potency in inhibiting mycelial growth of all isolated rhizosphere fungi of yellow pepper (*C.annum*.Var Nsukka yellow). This inhibition of mycelial growth of fungi by herbicide application is consistent with previous studies (Mohiuddin and Mohammed, 2014; Pakdaman and Goltapeh, 2014 and Wilkinson and Lucas, 1969). Although all the herbicides tested inhibited fungal growth at the recommended field application rates, it is worthy to note that none of the tested herbicides displayed fungal growth stimulation. Earlier, Zain et.al; (2013) showed that significant increase of fungal growth inhibition was observed with increasing herbicide concentration from 0.5x to 2x of their field recommended rates indicating a positive correlation between growth inhibition and treatment rates. Fungal growth inhibition due to the effect of herbicide treatments also varied among fungal species and type of herbicide.

Growth inhibition was pronounced on *Fusarium solani* than all fungi screened at almost all the concentrations of the herbicide treatments. This was followed by *Rhizoctonia solani* except for those treated with Primextra herbicide. Glyphosate, Paraquat, Atrazine and Linuron were found to be more potent in inhibiting the mycelial growth of all the rhizosphere fungi screened than Primextra. There were however, significant difference ($P \leq 0.05$) in the inhibition of the mycelial growth among different fungi at different concentrations of each herbicide tested. Toxicity of Glufosinate-ammonium (a herbicide) to fungus was reported by Tubajika and Daman (2002), where its high concentration (2000 µ*g*/ml) reduced the growth of *Aspergillus flavus* up to 80% and the same for *Trichoderma harziamu* and *T. longipilus* as was reported by Ahmad and Malloch, (1995). Other *In vitro* studies conducted on Glyphosate reported the growth inhibitory effects on *F.solani, Pythium altimum* and *Trichoderma virdae* at 100 and 140 ppm (Meriles et.al; 2006) and *Sclerotium rolfsii* at commercial recommended rate of 3.6g/litre (Westerhius et.al; 2007) Sushir and Pandey, (2001) reported that three herbicides – Fluchloralin , Oxadia zon and Pendimethalin adversely affected the growth of *Trichoderma* spp to the range of 42.22, 37.77 and 55.55 per cent even at 125 µ*g*/ml, 250µ*g*/ml and 500 µ*g*/ml respectively. Johnson et, al; (2002) also reported 48% growth inhibition of *Trichoderma* spp with Pendimethalin. However, Parakhia and Akbari, (2001) reported that six herbicides – Pendimethalin, Fluchloralin Butachlor, Paraquat, 2-4-D and Oxydiazon showed no adverse effect on the redial growth of *T.harzianum.* Shalaby et.al; (2002), stated that the fluctuations in the fungal populations indicate very wide range of sensitivity or tolerance of the soil fungi dependent on the various soil contaminants. El-Abyad et.al (1983) stated that biocide at any concentration caused a decrease in growth rate of fungi. They again stated that all the studied biocides caused inhibition of sporulation and germination of macroconidia of two form species of *Fusarium oxysperum.*

Herbicides could have different effects on soil microflora which could influence the microbial balance of soil which play vital role in soil fertility and crop yield. The present study revealed that there is a negative growth effect of herbicides on rhizosphere mycoflora of *C.annum* crop. These rhizosphere mycoflora which are adversely affected by these herbicides or other biocides are beneficial to the crop by the way of degrading the organic materials in the soil thereby making nutrients available to the crop.

CONCLUSION AND RECOMMENDATION

Based on the results obtained from this study, it is obvious that when herbicides are applied, the chemicals exert certain effects on non-target organisms particularly soil mycoflora. There is a negative effect of herbicides on common beneficial rhizospheric mycofloral organisms of pepper (*C.annum*). There is therefore need for the advert and use of cheaper, eco-friendly alternatives along with the judicious use of the known arsenal of agrochemicals. Further, it is necessary to strengthen the scientific basis of modern agriculture because herbicides may be advantageously used only if their persistent, bioaccumulation, and toxicity in agro-ecosystem are strictly controlled.

It is therefore recommended that any herbicide or biocide application to the soil should take into consideration of their effect on the numerous beneficial rhizosphere and rhizosplane mycofloral organisms. Herbicides should therefore be screened to know their biocidal effect on soil microflora before their application so that damage to the soil ecosystem can be prevented.

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