EFFECTS OF GLYPHOSATE AND NEEM SEED CAKE ADDITION ON MICROBIAL RESPIRATION AND UREASE ACTIVITY IN AN ULTISOL

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ABSTRACT

Application of organic amendments to soils could enhance microbial activity and reduce the inhibitory effect of herbicide. A laboratory incubation study was carried out to determine the effects of herbicide, (Force-Up with active ingredient of 480g l^{-1} Glyphosate- Isopropylamine salt) and neem seed cake (NSC) on soil microbial respiration and urease activity. Surface soil sample (0-15 cm) of Iwo Soil Series (Ultisol) was amended with neem seed cake (NSC) at the rates of 0, 2.5 and 5.0 t ha^{-1}*, respectively, and glyphosate herbicide was applied at the rates of 0 and 2.16 mg glyphosate/kg soil. There were six treatments replicated three times and arranged in a completely randomized design (CRD). The soil microbial respiration, assay of urease and pH were determined weekly starting from the 2nd week of incubation for a period of six weeeks. The results showed that the application of NSC solely or in combination with glyphosate significantly ($p<0.05$) increased soil microbial respiration, with 5* t ha^{-1} *leading to at least, 19 and 83% increases over the treatments with 2.5 and 0* t ha^{-1}*, respectively. While application of herbicide is mostly inhibitory, the NSC at the rates of 2.5 and 5* t ha^{-1} *without herbicide was better at enhancing soil urease activity. Further, NSC application improved soil pH, with higher performance at the earlier weeks of the incubation study. It was concluded that NSC application can reduce the inhibitory effect of herbicide on soil microbial activity, with the application rate of 5.0* t ha^{-1} *best at enhancing soil microbial respiration and urease activity.*

Keywords: amendment, neem seed cake, glyphosate, urease, microbial respiration

INTRODUCTION

The management of soil organic matter (SOM) - an important indicator of soil health and fertility is key to sustainable agriculture (Amiya *et al*., 2013). This is because, in addition to positively affecting soil physical and chemical properties, SOM, serves as a source of plant macro and micro nutrients, and of energy and cell carbon for soil microorganisms. Soil organic matter is replenished by materials of plant and animal origins such as plant remains, root exudates, dead animal tissues, cells of dead micro-organisms, manures etc. Agricultural and biodegradable industrial wastes are especially good sources of SOM, and consequently, of plant nutrients. An example of biodegradable industrial waste which has been used as bio-fertilizer or organic manure is neem seed cake (NSC). Neem seed cake is obtained as by-product of oil extraction from neem seeds by using high technology extraction methods such as cold pressing or other solvent extraction (NIIR Board, 2004; Chandy, 2013). It can be applied directly to soils, or prior to its application, can be mixed

with urea and other organic manure like farmyard manure and seaweed for best results (Subbalakshmi *et al.,* 2012). It is rich in nitrogen and phosphorus as well as sulphur, potassium, and calcium. NSC as an organic amendment is environmentally friendly, beneficial to soil microbial activity and thus, capable of improving the overall soil health and quality (Singh *et al*., 2006; Eifediyi *et al*., 2015).

Assessment of soil microbial activity is an important yardstick for measuring soil quality and fertility (Groffman *et al.,* 2001; Morugán-Coronado *et al.,* 2013). Microorganisms are capable of regenerating soil nutrients for the uptake of plant (Pandey *et al*., 2007). Soil microorganisms are also important in the degradation of herbicides and other toxic chemicals added to the soil (Benslama and Boulahrouf, 2013). There are many biochemical methods for measuring soil microbial activities (Sharma *et al*., 2006; Sartaj *et al.,* 2017). One of these is the measurement of microbial respiration, which indicates the activeness of soil microbial community and its decomposition of SOM. The amount of CO_2 evolved (microbial respiration) is an indicator of nutrients contained in organic matter being converted to forms available for plant uptake (Santos *et al.,* 2015). Another method of assessing soil microbial activity is the measurement of soil enzyme activities which are known to originate from soil microorganisms (Tabatabai, 1994). Enzymes are resident within the soil and play vital roles in the maintenance of soil ecology, its physical and chemical properties, and thus, its fertility and health (Das and Varma, 2011). Biochemical activities in the soil, such as degradation of organic compounds, and nutrient mineralization and recycling are driven by soil enzymes. Enzymes are quite sensitive and responsive to early changes in soil management, thereby making them a good index of soil fertility (Gianfreda *et al*., 2005; Truu *et al*., 2008; Garcia-Ruiz *et al.,* 2009). Urease is an extracellular enzyme that hydrolysis urea to ammonia and carbon dioxide. It plays a key role in the degradation of organic compounds, recycling, nutrient mineralization and regulates nitrogen supply to plants after urea fertilization (Makoi and Ndakidemi, 2008). The amount of urease enzyme indicates the biological activity of soil (Reddy *et al*., 2011). Urease activity in soil depends on the microbial community, physical and chemical properties of the soil, especially soil pH and temperature (Corstanje *et al*., 2007). There is a positive correlation between urease activity and microbial population in the soil (Pal *et al*., 2013). Urease enzyme is highly sensitive and is a useful indicator for evaluating soil pollution (Srinivasulu and Rangaswamy, 2014).

The activities of soil microorganisms and of the associated enzymes are influenced by the soil physico-chemical properties and ecological interactions such as with pesticides. In view of the need to sustain the rapidly growing world population, application of pesticides is important for securing improved crop yield and quality agricultural produce by keeping pests at bay (McDonald *et al.,* 1999). The shift from conventional to more sustainable conservation tillage practices with the adoption of herbicide-tolerant crop cultivars has further heightened the use of herbicides (Rose *et al.,* 2016). The use of herbicide has

been predicted to increase with the intensification of food production and urbanization (mostly in the developing countries) which cut on the available labor for manual weed control (Gianessi, 2013). However, the highest incidences of environmental contamination and deaths resulting from herbicide misuse have been recorded in the developing countries (including Nigeria) despite accounting for only 25% usage of the global herbicides production (Ojo, 2016). Often, only a fraction (sometime as small as 0.3%) of the applied herbicide reaches the target organisms (Pimentel, 1995). Thus, the residues become potential threat to the environment and public health through polluting soil, water, atmosphere, and food (Riaz *et al.,* 2007; Muñoz-Leoz *et al.,* 2011). The soil microbial diversity is also adversely affected (Latha and Gopal, 2010). Reports from authors vary from non-effect (Busse *et al*., 2001; Zabaloy and Gómez, 2008) to stimulation (Araújo *et al*., 2003; Eser *et al.,* 2007) of microbial respiration by glyphosate. Glyphosate was also reported to cause transient fluctuations in soil urease activity (Bello *et al*., 2013; Kucharski *et al*., 2016). However, because of the sorption capacity of organic matter on organic substances such as pesticide, application of organic fertilizer can reduce bioavailability of the applied pesticides (Muñoz-Leoz *et al.,* 2012). In a mesocosm experiment, Muñoz-Leoz *et al.* (2012) found that difenoconazole and deltamethrin caused a short-term inhibitory effect on microbial activity in non-fertilized soils, but not in fertilized soils. However, information is scarce on the effect of concurrent application of pesticide and neem seed cake on soil microbial activity in an ultisol. The objective of this study was to determine the effects of neem seed cake and herbicide (glyphosate) on soil microbial respiration and urease activity in an ultisol.

MATERIALS AND METHODS

Soil sampling and preparation of neem seed cake (NSC)

A topsoil (0-15 cm) of 'Iwo series' (ultisol) with no history of herbicide nor fertilizer application was obtained from an uncultivated field at the Obafemi Awolowo University Teaching and Research Farm, Ile-Ife, Osun State, Nigeria. The soil type was selected because of its sandy texture which due to its porous nature may enhance early response of soil microbial community to the applied herbicide and organic amendment. Also, the soil type, 'Iwo series' has the largest coverage in the south-western part of Nigeria. Several core samples of the soil were taken, homogenized, and taken to the laboratory in dark plastic bags. The fresh soil was gently crushed and sieved to <2 mm for the laboratory incubation experiment. A portion of the soil was air-dried for physical and chemical analysis. The NSC was obtained from the Department of Agricultural and Environmental Engineering, OAU, Ile-Ife. The NSC is a by-product obtained from cold pressing of neem seed and extraction of oil through solvent extraction process.

Soil physical and chemical analyses

Soil particle size distribution and water holding-capacity were determined by the hydrometer (Bouyoucos, 1962) and the core sampling methods (Veihmeyer and Hendrinkson, 1949), respectively. Soil pH was determined potentiometrically in a soil:

solution ratio of l:2 in 0.01 M $CaC1_2$ using EcoTestr pH 1 Waterproof pocket (Thomas, 1996). Exchangeable acidity was determined by the Mclean (1965) method. Exchangeable cations were determined by Helmke and Sparks (1996) method in which Calcium (Ca) and Magnesium (Mg) were determined by the Atomic Absorption Spectrophotometer (AAS) and potassium (K) and sodium (Na) by flame photometer. The organic carbon (OC) and total nitrogen (N) were determined by the chromic acid digestion method (Nelson and Sommers, 1996) and macro-Kjeldahl (Mulvaney, 1996), respectively.

Laboratory Incubation Experiment

Two hundred grammes (200 g) of soil was amended with NSC at the rate of 0, 2.5, and 5 t ha^{-1}, respectively, and poured into 500 ml incubation glass jars and maintained at 60% of its field moisture capacity. Glyphosate (Force-Up with active ingredient concentration of 480g l^{-1} Glyphosate-Isopropylamine salt) was applied to the samples at the rate of 2.16 mg glyphosate (active ingredient) kg^{-1} dry weight (DW) soil (Araújo *et al*., 2003). There were six treatments replicated three times and arranged in a completely randomized design. The treatments were:

1. Soil only (Control) (C_0 H_0)
2. Soil + 2.5 t NSC ha^{-1} (C_1H_0)
3. Soil + 5 t NSC ha^{-1} (C_2H_0)
4. Soil + Herbicide + 2.5 t NSC ha^{-1} (C_1H_i)
5. Soil + Herbicide + 5 t NSC ha^{-1} (C_2H_i)
6. Soil + Herbicides (C_0H_i)

Where C_0 = 0 t NSC $ha^{-1;}$ *C_1 = 2.5* t NSC ha^{-1}; *C_2 = 5* t NSC $ha^{-1;}$ *H_0 = without herbicide application; Hi = with herbicide application*

Ten millilitres (10 ml) each of 1 M NaOH was measured into vials and suspended in the incubation glass jars with the aid of lengths of thread and capped tightly after applying Vaseline (petroleum jelly) at the contact between the bottles and the lids to make them air tight. The 1 M NaOH was meant to capture the CO_2 evolved from the respiration of the soil microorganisms. The CO_2 released was determined weekly using the double acid titration method (Anderson, 1982) over a period of six (6) weeks, starting from the second week of incubation. Five (5) g of treated soil was weighed weekly through non-destructive sampling method into a 50 ml volumetric flask, 0.2 ml of toluene; 9 ml of THAM buffer and 1 ml of 0.2 M urea solution were added. The flasks were swirled, stoppered and incubated at 37 ^{0}C for 2 hours after which 35 ml of KCl-Ag_2SO_4 was added. For the control, addition of 1 ml of 0.2 M urea was done after the addition of 35 ml KCl-Ag_2SO_4 solution and made up to 50 ml mark after cooling. The incubated soil was sub-sampled weekly to determine the activity of urease enzyme by estimating the released ammonia through the steam distillation of the soils as described by Mulvaney (1996).

Data analyses

Statistical analysis was done using the SAS software package (SAS 9.0 version). The data collected were subjected to ANOVA technique, and means were separated using the Duncan's New Multiple Range Test (DNMRT) at 5% probability level.

RESULTS AND DISCUSSION

Physical and chemical properties of the soil and NSC used for the incubation studies

The result of the analysis of the soil and NSC used for the incubation studies are presented in Table 1. The textural classification of the soil was sandy, comprising 772, 38 and 190 g kg^{-1} sand, silt and clay, respectively. The field moisture capacity was 22%. The pH of the soil was 5.04 which is very strongly acidic to strongly acidic according to USDA classification. This could be due to removal of basic cations by the plants and/or leaching. The organic matter content (2.87%) was high according to classification of Adepetu *et al*. (2014), resulting from many years of accumulation of leafy litters which subsequently decompose and mineralize to yield organic matter (Olayinka, 2009). The total Nitrogen (0.88%) was high according to Adepetu *et al*. (2014). The critical minimum of 0.11% N is required for Nigerian soils (Adepetu *et al*., 2014). The exchangeable acidity was 1.84 cmol kg^{-1}. The exchangeable K^+ and Ca^{2+} with values of 0.23 and 2.58 cmol kg^{-1}, respectively, were low according to Adepetu *et al*. (2014). Hence, the soil was low in fertility. The NSC had a total N content of 4.62%, total P content of 0.65%, cation contents of 1.26% K, 37.41% Ca, 16.54% Mg and 1.11% Na. It was thus, a good source of energy to soil microbial population, and source of mineralizable nutrients to plant.

TABLE 1: PHYSICAL AND CHEMICAL PROPERTIES OF THE SOIL AND NSC USED FOR THE INCUBATION STUDIES

Properties	Soil Value	NSC value
Sand (g kg^{-1})	771.6	-
Silt (g kg^{-1})	38.2	-
Clay (g kg^{-1})	190.2	-
Textural class	Sand	-
FMC (%)	21.9	-
pH (H_2O)	5.23	7.60
pH (0.01 M $CaCl_2$)	5.04	7.43
Organic carbon (%)	2.87	7.35
Total N (%)	0.88	
Exchangeable cations (cmol kg^{-1})		
Ca	2.58	-
K	0.23	-
Mg	6.26-	-
Available P (mg kg^{-1})	27	-
Exchangeable Acidity (%)	1.84	-
Total K (%)	-	1.26
Total Mg (%)	-	16.54
Total Na (%)	-	1.11
Total P (%)	-	0.65
Total Ca (%)	-	37.41
Total N (%)	-	4.62

Effect of herbicide (glyphosate) and NSC on soil microbial respiration over a six (6) week incubation period

Microbial respiration as indicated by CO_2 evolution exhibited a decrease trend from the 2nd to the 6th week of incubation (Table 2). At the 2nd week of incubation, the CO_2 evolution in the glyphosate- and NSC-treated soil increased ($p<0.05$) with the applied rates of NSC. The presence of the amendment masked the inhibitory effect of the herbicide on soil microbial respiration. This is evident with the higher CO_2 evolution in the presence of NSC with or without herbicide (C_2H_i, C_2H_0) application. Conversely, the CO_2 evolution was the least at zero rate of NSC with or without herbicide (C_0H_i, C_0H_0) application. This trend of higher CO_2 evolution with higher rates of NSC with or without herbicide application continued till the 6th week of incubation with C_2H_i and C_2H_0 resulting in the highest ($p<0.05$) cumulative CO_2 evolution when compared with other treatments including the control. The results showed that herbicide application could hinder soil microbial activity. However, similar to previous studies (Muñoz-Leoz *et al.,* 2012), the added NSC could release organic compounds, stimulating the overall microbial activity and faster degradation of the pesticide. The NSC could also reduce bio-availability of the added pesticide via sorption (Barriuso *et al*., 1997). There is also the possibility of some sensitive microbial community being killed by the pesticide while the surviving microorganism mineralized the lysed cells (Cycon *et al*., 2010). The results, nevertheless, contrasted with those of Ratcliff *et al*. (2006) who reported transient increase in fungal propagules and culturable bacteria after Glyphosate addition at the rate of 50 mg kg^{-1}. The result was attributed to the glyphosate acting as a source of N to soil microorganisms.

TABLE 2: MICROBIAL RESPIRATION IN SOIL TREATED WITH HERBICIDE (GLYPHOSATE) AND NSC OVER SIX (6)-WEEKS INCUBATION PERIOD (MG C/100G SOIL).

	Incubation period (weeks)					
TREATMENT	2	3	4	5	6	Cum CO_2
C_0H_0	8.75c	2.20c	2.55c	2.25b	1.35bc	17.10c
C_0H_i	9.65c	1.95c	2.55c	1.60c	1.20c	16.95c
C_1H_0	16.10b	3.30bc	3.20bc	1.95bc	1.65abc	26.20b
C_1H_i	15.80b	3.10bc	3.15bc	2.10bc	1.75ab	25.90b
C_2H_0	17.45ab	6.65a	4.15a	2.95a	2.15a	33.35a
C_2H_i	18.70a	4.45b	3.75ab	2.40b	1.95a	31.25a

Means in a column with the same letter(s) are not significantly different ($p<0.05$) according to Duncan's New Multiple Range Test. Where, C = NSC; H = Herbicide; 0 = No addition; 1 = 2.5 t NSC ha^{-1}; 2 = 5 t NSC ha^{-1}; i =2.16 mg glyphosate/ kg soil.

Effects of glyphosate and neem seed cake on urease activity in soil over six (6) week incubation period

The soils treated with sole NSC (C_2H_0 and C_1H_0) had higher urease activity than those treated with combined NSC and glyphosate or glyphosate only, over the six (6)-week incubation period (Table 3). The stimulatory effect of the applied NSC on urease activity, however, declined with the increasing length of incubation, probably due to gradual depletion of nutrient supplied by the NSC with the length of incubation. The highest ($p<0.05$) mean urease activity was obtained with sole application of 5.0 t NSC ha^{-1} while the application of glyphosate had obvious inhibitory effect on soil urease activity, except when applied with 5.0 t NSC ha^{-1}. This result is in agreement with earlier report of higher urease activity in soils with, than without nutrient application (Vandana *et al*., 2012). The organic amendment, NSC, could stimulate increased abundance of microorganisms releasing the urease enzyme into the soil by providing the needed energy for growth, and carbon for the formation of new cells (Lalfakzuala *et al.*, 2006; Balezentiene and Kilimas, 2009). Herbicide application on the other hand could result in decrease of microbial population especially bacteria (Sebiomo *et al*., 2011). Thus, excessive application of herbicide can inhibit soil microbial activity, and by extension, soil quality, especially under low organic inputs.

TABLE 3: UREASE ACTIVITY (µG NH-N G^{-1} DWT $2H^{-1}$) IN SOILS TREATED WITH GLYPHOSATE AND NEEM SEED CAKE OVER SIX (6) WEEK INCUBATION PERIOD

Treatments	Incubation period (weeks)					Mean Urease
	2	3	4	5	6	
C_0H_0	80.83^{b}	107.78^{bcd}	94.38^{b}	86.89^{a}	52.26^{b}	84.43d
C_0H_i	65.68^{b}	70.35^{d}	107.78^{b}	85.55^{a}	70.73^{ab}	80.02d
C_1H_0	124.62^{b}	138.09^{ab}	138.09^{a}	92.96^{a}	84.20^{a}	115.59b
C_1H_i	95.99^{b}	91.00^{cd}	84.20^{b}	94.98^{a}	74.10^{ab}	88.05d
C_2H_0	232.38^{a}	165.03^{a}	134.72^{a}	100.37^{a}	84.20^{a}	143.34a
C_2H_i	77.46^{b}	124.62^{abc}	97.67^{b}	109.12^{a}	77.46^{ab}	97.27c

Means in a column with the same letters are not significantly different ($P < 0.05$) by DNMRT. Where, dwt = dry weight; C = NSC; H = herbicide; 0 = no addition; 1 = 2.5 t NSC ha^{-1}; 2 = 5 t NSC ha^{-1}; i = 2.16 mg glyphosate / kg soil.

Effect of herbicide and neem seed cake application on soil reaction (pH 0.01 M $CaCl_2$)

The pH values ranged from 5.37 to 6.77 which was within the strongly acidic to neutral range by USDA classification (Table 4). The soil pH was significantly ($p<0.05$) increased by C_2H_0 over the control at 2nd week of incubation. Addition of NSC with or without glyphosate (C_2H_i, C_2H_0, C_1H_i and C_1H_0) also increased the soil pH at the third week of incubation when compared with

treatment without NSC addition. However, subsequently, from the fourth week, the treatments were not significantly different from one another probably due to drastic reduction in the organic material as it was degraded by microorganisms.

The positive effect of the NSC on soil pH could enhance soil microbial activity (respiration and urease) as observed in this study. The significant linear relationship (R^2 = 0.92, $p < 0.01$) between the urease activity and soil pH (Table 5) is an indication that pH can negatively or positively affect soil microbial activity. This result was similar to earlier reports of improved soil pH with organic amendment attributable to the high Ca and Mg in the organic amendment (Naramabuye and Haynes, 2006), and the positive relationship between soil pH and urease activity (Baath and Anderson, 2003).

TABLE 4: SOIL REACTION (PH 0.01 M $CACL_2$) AS AFFECTED BY TREATMENT OF SOIL WITH HERBICIDE AND NEEM SEED CAKE.

Incubation period (weeks)						
TREATMENT	2	3	4	5	6	Avr pH
C_0H_0	5.63b	5.43b	5.57a	5.57a	5.70a	5.58a
C_0H_i	5.67b	5.37b	5.53a	5.60a	5.73a	5.55a
C_1H_0	6.03ab	5.60a	5.47a	5.70a	5.67a	5.69a
C_1H_i	5.50b	5.57a	5.47a	5.53a	5.57a	5.53a
C_2H_0	6.77a	5.63a	5.47a	5.73a	5.57a	5.83a
C_2H_i	6.33ab	5.63a	5.50a	5.70a	5.43a	5.72a

Means in a column with the same letter(s) are not significantly different ($p<0.05$) according to Duncan's New Multiple Range Test. Where, C = NSC; H = Herbicide; 0 = No addition; 1 = 2.5 t NSC ha^{-1}; 2 = 5 t NSC ha^{-1}; i =2.16 mg glyphosate/ kg soil.

TABLE 5: CORRELATION AMONG PARAMETERS

	Mean cum CO2	Mean pH
Mean urease	0.68**	0.92**
Mean cum CO2	-	0.77

CONCLUSION

Application of neem seed cake to soil can ameliorate the soil pH which is beneficial to improved soil microbial activity. Although application of NSC with or without herbicide are beneficial, NSC applied at the rate of 5.0 t ha^{-1} proved superior to other treatments at enhancing soil microbial respiration and soil urease activity. Thus, application of organic amendment such as NSC at sufficient rates can reduce the negative impact of herbicide on soil microbial activity, especially under conventional farming system involving intensive use of pesticides.

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