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SOYBEAN NODULATION AND N_2-FIXATION AS INFLUENCED BY COW DUNG, WOOD ASH AND RHIZOBIAL INOCULATION IN TROPICAL ACID SOILS (TYPIC DYSTRUDEPT)

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ABSTRACT

The effects of cow dung and wood ash applications with and without rhizobial inoculation on modulation, and N_2-fixation by soybean *(Glycine max* (L.) Merrill) were investigated in tropical acid dystrudepts under greenhouse and field conditions. The field experiments were located at Epe and Ijebu-waterside (Ibiade) of Lagos and Ogun States, southwestern Nigeria, respectively. The experiment was a 3 x 3 x 2 factorial experiment, arranged in randomized complete block design (RCBD) with three replications. The treatments were three rates (0, 5, and 10 t ha^{-1}) of cow dung, three rates (0, 2.5 and 5.0 t ha^{-1}) of wood ash and two levels of inoculation (inoculated and un-inoculated). Inoculation significantly ($p < 0.05$) increased the number of nodules and the N-fixed by soybean. The increase in the numbers of nodules of the inoculated over the un-inoculated soybean under field conditions at Epe and Ijebu-waterside were 76% and 98% respectively. The amount of nitrogen fixed was found to be greatest at 5 t ha^{-1} of both cow dung and wood ash in the greenhouse. The soil pH increased with the increasing rate of cow dung and wood ash applications at the two locations. The maximum value of pH (7.08) was obtained at the application rate of 5 t ha^{-1} wood ash singly or combined with 10 t ha^{-1} cow dung. In the greenhouse, the grain yield increases of the un-inoculated and the inoculated soybean over the control were 39 and 86% in the soil of Epe, and 67 and 112% in the soil of Ijebu-water-side, respectively. It is concluded that combination of cow dung and wood ash applications with rhizobial inoculation improved nodulation and N_2-fixation by soybean in acid soils.

Key words: Cow dung, N_2-fixed, rhiozobial-inoculation, soybean, wood ash.

INTRODUCTION

Generally, N fertilizer application is not recommended for leguminous crops, however, it is always advocated that organic manure should be applied along with rhizobium culture. Although organic manure does not take part directly in N_2 fixation, reports available have indicated that the incorporation of organic manures into the soil improved the soil physical condition and provided favourable environment for the proper establishment of rhizobium and the growth and development of nodules for N_2 fixation (Fried *et al.*, 1983). Olayinka *et al.* (1998) also found that organic amendments could benefit rhizobia, which are heterotrophs, and N_2

fixation, especially in soils low in indigenous organic matter.

Although, the influence of organic wastes on cereal crops is well known (Olayinka and Adebayo, 1984; 1989), not much work has been done in relation to legumes especially with regard to symbiotic N_2 fixation. Olayinka (1900) observed that liming a poultry manure amended acidic ultisol enhanced microbial decomposition of the organic manure, and the release of NO_3-N and available P. Hence, liming along with organic manure would be beneficial to plants grown in an acidic ultisol. Board and Caldwell (1991) hypothesized that a major effect of soil acidity on soybean yield was a reduction in the ability of the plants to fix N_2, either by interfering with nodulation or reducing the availability of molybdenum (Mo).

The most commonly used liming materials are the oxides, carbonates and silicates of calcium and magnesium. Only scanty information is available on miscellaneous materials such as shells, industrial slag, flue-dust from cement, iron smelting slag, chicken litter ash and wood ash. The liming value and phosphate fertilizer effectiveness of these materials are poorly defined. They are byproducts which are commonly dumped in landfill; therefore, any use of byproducts to ameliorate land will contribute to the preservation of alternative resources and provide an effective solution for the disposal of byproducts. This study was therefore conducted with the aim of investigating the effects of the applications of cow dung and wood ash, with and without rhizobial inoculation on the N_2-fixation by soybean in acid soils.

MATERIALS AND METHODS

The experiment was carried out in two phases. The first phase was a greenhouse study, at the University of Agriculture, Abeokuta (UNAAB), located within the transitional zone of the sub-humid rainforest to the south and the derived savannah to the north and west. It lies between the latitude 7^{o}1 North and longitude 3^{o}3′ East in the southwest of Nigeria. The study was conducted with two acid soils from Epe and Ijebu-Waterside areas of Lagos and Ogun States, Nigeria respectively. The second phase was a field trial which was conducted at Epe and Ijebu-Waterside areas of Lagos and Ogun States, respectively. Epe is the rain forest and lies between Lat 6^{o}59′N and Long 3^{o}59′E while Ijebu-Waterside lies between Lat. 6^{o}55′N and Long. 4^{o}45′N.

Soil Sampling and Preparation

Representative initial soil samples were collected randomly at 0-15 cm depth from the two locations, air-dried and passed through a 2 mm sieve. The soil obtained at Epe soil is a sandy loam with a pH of 5.3 while that obtained at Ijebu-Waterside is loamy sand with a pH of 5.5. Both soils are classified a Typic Dystrudepts according to Soil Survey Staff (1999).

Sources of Materials Used and Preparation

The rhizobium inoculants used was sourced from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife. Osun State. The cow dug used was obtained from the Animal Research Farm, University of Agriculture, Abeokuta, Ogun State.

Wood ash was sourced from a commercial bakery in Abeokuta. The soybean seed used (TGx 1448-2E) was obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State. Cow dung and wood ash were sun-dried and passed through a 2 mm sieve before application.

Soil Physical and Chemical Analyses

The experimental soil, cow dung and wood ash samples were analyzed routinely for pH, organic matter, exchangeable Ca, Mg, K and Na, total K, available P, and particle size distribution. The soil pH was determined potentiometrically in water (1:2 soil; solution), organic carbon was determined using the chromic acid digestion method of Walkley and

Black as modified by Sparks (1996). The total N concentration was determined using macro-Kjeldahl method of Bremner (1996). Available P was extracted using Bray-l method as described by Kue (1996). Exchangeable K, Ca, Na and Mg were extracted with neutral solution of 1 N ammonium acetate (NH_4OAc) buffer. Potassium, Na and Ca K were determined using flame photometer and exchangeable Ca and Mg with atomic absorption spectrophotometer (AAS). Particle size distribution was determined by the hydrometer method (Bouyoucos, 1962) using hexametaphosphate as the dispersing agent. The field moisture capacity (FMC) was determined according to Hanks *et al*. (1954).

Experimental Procedures

The soil culture experiment was a 3 x 3 x 2 factorial, with completely randomized design. The treatments were three rates (0, 5 and 10 t ha^{-1}) each of cow dung, and wood ash and two levels of inoculation (inoculated and un-inoculated). The resulting 18 treatments were replicated three times. Five kilogramme portions of soil were weighed into each plastic pot, mixed thoroughly with the treatments and moistened with water to 70% Field Moisture Capacity. This was left for about two weeks before planting. The soybean seeds were inoculated with rhizobium inoculants before planting. Four seeds were planted per pot.

The field study was conducted at two locations, namely Epe and Ijebu-Waterside in Lagos and Ogun States of southwestern Nigeria, respectively. The greenhouse experiment was repeated in the field with the treatments arranged in a randomized complete block design (RCBD) with three replications. The plot size was 2 m with 0.5 m alley between plots and 1 m between replicates. The treatments were applied and allowed to equilibrate for two weeks before planting. The soybean seeds were inoculated with rhizobium inoculants and planted by seed drilling with inter-row spacing of 0.5m. The seedlings were thinned to an intra-row spacing of 0.05m at two weeks after planting. Surface soil samples (0-15 cm) were collected at 8 weeks after planting (WAP), air-dried, passed through 2 mm sieve and analyzed for pH, organic carbon and total N (Page *et al.*, 1982). Plant samples were also collected at 8 WAP and analysed for N-fixed by ureide assay (Hot water Extraction) and the number of nodules per plant. Grain yield was also determined at harvest.

Some physical and Chemical Properties of the Experimental Soils and Amendments

The characteristics of the soil and amendments used for the study are presented in Table 1. The soil from Epe was loamy sand, strongly acid in reaction (pH 5.3), low in organic carbon content (5.4 g/kg^{-1}), available P (13.2 mg/kg^{-1}) and total N (0.9 g/kg^{-1}). The soil was low to medium in exchangeable cations with values of 2.78, 1.21, 0.42 and 0.93 cmol kg^{-1} for Ca, Mg, K and Na respectively. Hence, the soil was low in fertility.

The soil from Ijebu-Waterside was loamy sand with contents of sand, silt and clay being 90, 4 and 6% respectively. It was strongly acid in reaction (pH 5.5) and high in organic carbon (20.8g/kg^{-1}). The soil was moderate in total N (1.8g/kg^{-1}), low in available P (24 mg kg^{-1}) and low in exchangeable K^+, Ca^{2+} and Na^+ with values of 0.20, 0.62, 0.31 and 3.66 cmol kg^{-1} respectively. Both soils are classified as Typic Dystrudepts (Soil Survey Staff, 1999).

Statistical Analysis

Statistical analyses were done using SAS (Statistical Analysis System Institute Inc., 1990). The experimental data collected were subjected to analysis of variance (ANOVA) to determine the statistical differences between treatments at 5% probability. The treatment means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS

Greenhouse Experiment

Single Treatment Effects on the Soil pH

Wood ash (W) significantly ($P<0.05$) increased soil pH with increasing rates of application at 4 WAP (Table 2). At 8 WAP, on the other hand, the values were the same at 2.5 and 5 t ha^{-1} but were significantly ($P<0.05$) higher than in the control. Wood ash also had significant ($P<0.05$) effect on the pH of the soil from Ijebu-waterside at both 4 and 8 WAP. The trend showed that, as the wood ash application rates increased, the pH of the soil also increased, meaning that the acidity of the soil was reduced. Hence, the pH was highest at 5 t ha^{-1} and lowest in the control. The pH of the soil from Ijebu-water was about 5% higher than that from Epe at 5.0 t ha^{-1} W application. Cow dung (C) on the other hand, did not significantly ($P>0.05$) affect the pH of both soils at 4 and 8 WAP.

Combined treatment effects on the soil pH

Cow dung and wood ash significantly ($P<0.05$) affected the soil pH at all the stages of plant growth in both soils (Table 3). Cow dung and wood ash had significant ($P<0.05$) effects on the pH of both soils at 4 and 8 WAP. For the soil from Epe, the pH was highest at C_5 W_5 (5 t ha^{-1}) of both C and W) and lowest at C_5 W_0 (5 t ha^{-1} of C only) at 4 WAP. At 8 WAP, the highest pH (6.27) was observed at C_0 W_5 (5 t ha^{-1} W application only) while the least was observed at C_5 W_0 (5 t ha^{-1} of C application only).

The soil of Ijebu-waterside followed similar pattern observed in the soil of Epe. At 4 WAP, the highest pH was obtained at C_0 W_5 and also at C_{10} W_5. The lowest pH was in the control and with C_0 W_{10}. Generally, a progressive increase in soil pH was observed at all levels of C applications with increasing rate of W application. At 8 WAP, the highest pH (7.08) was obtained with C_0 W_5 while the lowest was with C_5 W_0. The soil pH (6.87) obtained at C_5 W_5 and C_0 $W_{2.5}$ was not significantly different from the highest pH.

A general increasing trend in pH was observed at 0 and 5 t ha^{-1} C applications as the W application rates increased. At 10 t ha^{-1} C application, an increase in pH was later followed by a decrease as the W application rates increased.

Soybean Nodulation in the Greenhouse

Figure 1 shows the combined effects of C and W on the nodulation of un-inoculated and inoculated soybean sown in the soil of Epe and Ijebu-waterside.

In the soil of Epe, the number of nodules varied significantly with respect to the treatment combinations. Among the un-inoculated soybean, the highest number of nodules was obtained at C_{10} W_5 followed by C_0 W_5 > C_5 W_0 = C_{10} W_0 = C_{10} W_0 > C_5 W_5 = C_{10} $W_{2.5}$ > C_0 $W_{2.5}$ > C_5 $W_{2.5}$ in the decreasing order. It was further observed that at 0 and 5 t ha^{-1} of W applications, the number of nodules increased as the rate of C was increased. The inoculated soybean also varied significantly in nodulation with respect to the combined applications of C and W. The highest number of nodules was also obtained at C_{10} W_5 as observed in the un-inoculated soybean, this as followed by C_{10} W_0 > C_0 W_5 = C_0 $W_{2.5}$ > C_{10} $W_{2.5}$ = C_5 W_5 > C_5 W_0 > C_0 W_0 > C_5 $W_{2.5}$ in the decreasing order. The pattern of response observed was similar to that of the un-inoculated soybean, in which the number of nodules increased as the rate of C was increased at 0 and 5 t ha^{-1} of W applications. The number of nodules also increased as the W rate increased especially without C application. The per cent increase of the highest number of nodules by the inoculated and the un-inoculated soybean over the control were 167 and 242% respectively. The percent increase of the highest number of nodules by the inoculated over that of the inoculated soybean was 231%.

In the soil of Ijebu-waterside, the number of nodules from the un-inoculated soybean was highest at C_{10} W_5 and lowest in the control (C_0 W_0). The pattern followed the order C_{10} W_5 > C_0 W_5 > C_5 W_5 = C_{10} $W_{2.5}$ > C_{10} W_0 = C_5 W_0 > C_5 $W_{2.5}$ > C_0 $W_{2.5}$ > C_0 W_0. At both 0 and 10 t ha^{-1} of cow dung, there was a general increase in number of nodules as the wood ash application rate was increased. The inoculated soybean had the highest number of nodules with C_0 W_5 and the lowest at C_5 $W_{2.5}$. The nodulation pattern followed the order C_0 W_5 > C_{10} W_5 > C_{10} $W_{2.5}$ > C_5 W_5 > C_0 $W_{2.5}$ > C_{10} W_0 = C_5 W_0 = C_0 W_0 > C_5 $W_{2.5}$. A progressive increase in the number of nodules was observed as the wood ash rate was increased at each level of C application except at 5 t ha^{-1}.

Generally, the numbers of nodules produced by the inoculated soybean were consistently higher than those of the un-inoculated in both soils at every treatment combination.

Combined treatment effects on the N-fixed by inoculated and un-inoculated soybean

Figure 2 shows the combined effects of cow dung and wood ash on the N-fixed by the inoculated and un-inoculated soybean sown in soil of Epe. Cow dung and wood ash significantly (P<0.05) affected the N-fixed by the inoculated and un-inoculated soybean. The inoculated soybean was highest in N-fixed at C_5 W_5 and C_{10} W_5, while the lowest was obtained in the control. All the other treatment combinations were not significantly (P<0.05) different from the treatments that gave the highest N-fixed except C_0 $W_{2.5}$. Apart from C_5 W_5 and C_{10} W_5 that gave the highest N-fixed, C_5 W_0 and C_0 W_5 also gave significantly higher N-fixed than the control.

The un-inoculated soybean, on the other hand, was highest in N-fixed at C_5 W_5 (5 t ha^{-1} of both C and W applications) and lowest at C_5 $W_{2.5}$ (5 and 2.5 t ha^{-1} of C and W applications, respectively). All the other treatment combinations were not significantly (P<0.05) different from each other. The N-fixed by the inoculated soybean were significantly (P<0.05) higher than the un-inoculated only at C_5 W_0, C_5 $W_{2.5}$ and C_{10} W_5. All the other treatment combinations were not significantly (P<0.05) different. The per cent increase of the highest N-fixed by the inoculated and the un-inoculated soybean over the control were 10 and 7% respectively.

Figure 3 shows the effect of the combined application of C and W on the N-fixed by inoculated and un-inoculated soybean sown in soils of Ijebu-waterside. In the soil of Ijebu-waterside, the N-fixed by the inoculated soybean was highest at C_5 W_5 and lowest at C_5 W_0. All the other treatment combinations were not significantly (P<0.05) different from each other. The un-inoculated soybean, on the other hand was highest at C_0 $W_{2.5}$ and lowest at the varying combinations of C with zero rates of W. The significance of wood ash in the N-fixed by soybean sown in soil of Ijebu-waterside is therefore implied. The N-fixed by the inoculated and the un-inoculated were significantly different only at C_0 W_0, C_0 $W_{2.5}$, C_{10} W_0, C_{10} $W_{2.5}$ and C_{10} W_5. The inoculated soybean was, however, generally higher in N-fixed than the un-inoculated except at C_0 $W_{2.5}$ and C_{10} $W_{2.5}$. The increase of the highest N-fixed by the inoculated and the un-inoculated soybean over the control were 9 and 22% respectively. The N-fixed by soybean in the soil of Epe were not significantly different with respect to the treatment combinations.

Combined treatment effects on the dry grain yield of inoculated and un-inoculated soybean

Figure 4 shows the combined effects of cow dung and wood ash on the grain yield of inoculated and un-inoculated soybean sown in the soil of Epe in the greenhouse. The grain yields were significantly (P<0.05) affected by the C and W applications. In the soil of Epe, the grain yield of the un-inoculated soybean was found to be highest at C_0 $W_{2.5}$ and lowest at C_5 W_0. All the other treatment combinations were not significantly (P<0.05) different.

Although the grain yields were not significantly different in the inoculated soybean, the treatment C_5 W_5 appeared to give the highest grain yield.

The highest grain yield in the inoculated and the un-inoculated compared with the control gave an increase of 39 and 86% respectively. On the other hand, the highest grain yield of the inoculated soybean at C_5 W_5 compared with the un-inoculated counterpart gave an increase of 29%.

Figure 5 shows the combined effects of cow dung and wood ash on the grain yield of inoculated and un-inoculated soybean sown in the soil of Ijebu-waterside. In the soil of Ijebu-waterside, the grain yields of the inoculated soybean were significantly ($P<0.05$) affected by C and W applications. The highest grain yield was obtained at C_0 W_5 while the lowest yield was at C_5 $W_{2.5}$. The highest grain yield at C_0 W_5 was however, not significantly different from C_5 W_5 which was next to it in value. It was further observed that in the absence of wood ash (i.e. at 0 t ha^{-1}), there was an initial increase in yield (i.e. at C_5) which later declined as the C application increased to 10 t ha^{-1}. A similar pattern was also observed with W_5. In other words, grain yield was maximum at the combinations of W (0 and 5 t ha^{-1}) with C_5. The amendments also had significant ($P<0.05$) effect on grain yield of the un-inoculated soybean at harvest. The highest grain yield was obtained with C_0 W_5 while the lowest was obtained with C_5 $W_{2.5}$. The highest grain yield at C_0 W_5 was also not significantly different from C_5 W_5 which was next to it in value. All the other treatment combinations were not significantly different.

The highest grain yield was obtained with the inoculated soybean at C_0 W_5 and C_5 W_5 and these amounted to about 145 and 132% increases respectively, compared with the control. When these grain yield values were compared with the un-inoculated counterparts at C_0 W_5 and C_5 W_5, the yield increases amounted to 112 and 67% respectively.

Field Experiments

Single treatment effects on the soil pH

Table 4 shows the effects of cow dung and wood ash on the soil pH under field conditions at Epe and Ijebu-waterside. At Epe, cow dung and wood ash had significant ($P<0.05$) effects on the soil pH. Wood ash application significantly ($P<0.05$) increased the soil pH over the control. Although there was no significant ($P>0.05$) difference between 2.5 and 5.0 t ha^{-1} application rates, the trend, however, showed an increase as the level of application increased. At Ijebu-waterside, the soil pH was not significantly ($P>0.05$) affected by the cow dung application. Wood ash application, on the other hand significantly ($P<0.05$) affected the soil pH. It was lowest in the control with the highest pH obtained at 5.0 t ha^{-1} W applications. There was, however, no significant ($P > 0.05$) difference in soil pH at 2.5 and 5.0 t ha^{-1} W applications.

Combined treatment effects on the nodulation soybean under field conditions

The number of nodules differed significantly with respect to the treatment combinations (Figure 6) under the field conditions. Among the un-inoculated soybean, the highest number of nodules was obtained at C_{10} $W_{2.5}$ and the lowest at the control (C_0 W_0). The nodulation pattern followed the order C_{10} $W_{2.5}$ > C_0 W_5 > C_5 W_5 > C_{10} W_5 > C_0 $W_{2.5}$ > C_{10} W_0 = C_5 W_0 > C_0 W_0. It was further observed that the number of nodules increased as the rate of W increased in the absence C application i.e. at zero rate of C application. On the other hand, there was a steady decrease in the number of nodules as the C application increased at 5 t ha^{-1} W application.

The inoculated soybean also differed significantly in nodulation with respect to the combined applications of C and W. The highest number of nodules was also obtained at C_{10} W_5 as observed in the un-inoculated soybean, this was followed by C_{10} W_0 > C_0 W_5 = C_0 $W_{2.5}$ > C_{10} $W_{2.5}$ = C_5 W_5 > C_5 W_0 > C_0 W_0 > C_5 $W_{2.5}$ in the decreasing order. The pattern of response observed was similar to that of the un-inoculated soybean, in which the number of nodules increased as the rate of C was increased at 0 and 5 t ha^{-1} of W applications. The number of nodules also increased as the W rate increased especially without C application. The increased of the number of nodules of the inoculated over the un-inoculated soybean in the plot which produced highest number of nodules was about 75%.

At Ijebu-waterside field, the number of nodules per plant varied significantly with the combined application of cow dung and wood ash to the inoculated and un-inoculated soybean. In the un-inoculated soybean, the number of nodules was highest at C_0 W_5 and lowest in the control C_0 W_0. The pattern followed the order C_0 W_5 > C_5 W_5 = C_{10} W_0 > C_0 $W_{2.5}$ > C_{10} $W_{2.5}$ > C_5 $W_{2.5}$ > C_5 $W_{2.5}$ > C_{10} W_5 > C_5 W_0 = C_0 W_0. At 0 and 5 t ha^{-1} of cow dung application, there was a general increase in the number of nodules as the W application rate increased. The inoculated soybean also differed significantly in the number of nodules with respect to C and W applications. The highest number of nodules was obtained with C_{10} W_5 and the lowest with C_5 $W_{2.5}$. the pattern of nodulation followed the order C_{10} W_5 > C_0 $W_{2.5}$ > C_{10} $W_{2.5}$ > C_5 W_5 > C_{10} W_0 = C_5 W_0 = C_0 W_0 > C_0 W_5 > C_5 $W_{2.5}$. A general increase in the number of nodules was observed only at 10 t ha^{-1} C, as the W application rate increased. The increase of the number of nodules of the inoculated over the un-inoculated soybean in the plot which produced highest number of nodules was about 98%. Generally, the numbers of nodules recorded at Ijebu-waterside in the inoculated plots were higher than those at Epe.

On the other hand, there was a steady decrease in the number of nodules as the C application increased at 5 t ha^{-1} W application. The inoculated soybean also differed significantly in nodulation with respect to the combined applications of C and W. The highest number of nodules was also obtained at C_{10} W_5 as observed in the un-inoculated soybean, this was followed by C_{10} W_0 > C_0 W_5 = C_0 $W_{2.5}$ > C_{10} $W_{2.5}$ = C_5 W_5 > C_5 W_0 > C_0 W_0 > C_5 $W_{2.5}$ in the decreasing order. The pattern of response observed was similar to that of the un-inoculated soybean in which the number of nodules increased as the rate of C was increased at 0 and 5 t ha^{-1} of W applications. The number of nodules also increased as the W rate increased especially without C application. The increase of the number of nodules of the inoculated over the un-inoculated soybean in the plot which produced highest number of nodules was about 76%.

At Ijebu-waterside field, the number of nodules per plant varied significantly with the combined application of cow dung and wood ash to the inoculated and un-inoculated soybean. In the un-inoculated soybean, the number of nodules was highest at C_0 W_5 and lowest in the control C_0 W_0. The pattern followed the order C_0 W_5 > C_5 W_5 = C_{10} W_0 > C_0 $W_{2.5}$ > C_{10} $W_{2.5}$ > C_5 $W_{2.5}$ > C_5 $W_{2.5}$ > C_{10} W_5 > C_5 W_0 = C_0 W_0. At 0 and 5 t ha^{-1} of cow dung application, there was a general increase in the number of nodules as the W application rate increased. The inoculated soybean also differed significantly in the number of nodules with respect to C and W applications. The highest number of nodules was obtained with C_{10} W_5 and the lowest with C_5 $W_{2.5}$. The pattern of nodulation followed the order C_{10} W_5 > C_0 $W_{2.5}$ > C_{10} $W_{2.5}$ > C_5 W_5 > C_{10} W_0 = C_5 W_0 = C_0 W_0 > C_0 W_5 > C_5 $W_{2.5}$. A general increase in the number of nodules was observed only at 10 t ha^{-1} C, as the W application rate increased. The increase of the number of nodules of the inoculated over the un-inoculated soybean in the plot which produced highest number of nodules was about 98%. Generally, the numbers of nodules recorded at Ijebu-waterside in the inoculated plots were higher than those at Epe.

Combined treatment effects on N-fixed by soybean under field conditions

The N-fixed by the inoculated soybean was not significantly affected ($P > 0.05$) by the C and W application at Epe location (Table 5). There was, however, a general initial apparent decrease and eventual increase in N-fixed at all levels of 0 and 2.5 t ha^{-1} W applications as the C application increased except at 5 t ha^{-1} W application where a progressive apparent increase in N-fixed was observed. At Ijebu-waterside, the N-fixed was highest at C_{10} W_5 and lowest in the control. The percent increase of the highest N-fixed over the control was 39%.

The N-fixed by the un-inoculated soybean were significantly ($P<0.05$) affected by the application of C and W only at Ijebu-waterside field. The N-fixed was highest at C_0 $W_{2.5}$ and lowest in the control. The percent increase of the highest N-fixed over the control was 28%. The N-fixed also appeared to be generally higher at Ijebu-waterside location than that obtained at Epe.

Combined treatment effects on the grain yield of soybean under field conditions

Figure 9 shows the combined effects of C and W applications on the grain yield of inoculated and un-inoculated soybean at Epe and Ijebu-watersied. At Epe, the amendments had significantly ($P<0.05$) effect only on the grain yields of the inoculated soybean. The grain yield of the inoculated soybean was highest at C_0 $W_{2.5}$ and C_5 $W_{2.5}$ while the lowest was in the control. All the other treatment combinations were not significantly different. The un-inoculated soybean grain yields were not significantly ($P > 0.05$) affected by the applications of C and W. However, the response still followed similar pattern observed in the inoculated soybean. The increase of the highest grain yield from the inoculated and the un-inoculated plots compared with the control were 120 and 65% respectively. At Ijebu-waterside on the other hand, C and W did not significantly affect the grain yields of both inoculated and the un-inoculated soybean. However, the grain yield was apparently highest at C_5 W_5. The increase of the highest grain yield from the inoculated and the un-inoculated plots compared with the control were 33 and 56% respectively. When the grain yield from the inoculated plot was compared with the un-inoculated, increases of 4 and 16% were obtained at Epe and Ijebu-waterside, respectively.

DISCUSSION

Treatment affects on the soil pH

In both soils of Epe and Ijebu-waterside, the soil pH increased generally with increasing rates of W application. This is probably due to the increase in base saturation of the soil as a result of wood ash addition. Wood ash is relatively high in exchangeable bases, hence, the more wood ash added, the more the base saturation of the soil. Various authors had earlier observed a significant increase in soil pH as a result of lime additions (Ojeniyi, 2007; Baath *et al.*, 1980; Lang and Beese, 1985). Ojeniyi (2007) found an increase in soil pH after the addition of wood ash. Baath *et al*., (1980) found an increase in nitrification owing to an increased pH occasioned by lime addition to soil, Lang and Beese (1985) on the other hand, found that an increase in pH due to lime addition favoured an increased in microbial population. The highest pH obtained in the soil of Ijebu-waterside over that of Epe may be probably due to the initial higher pH of the soil, and the probable lower buffering capacity of the soil than that of Epe. The soil of Ee is higher in clay content than that of Ijebu-waterside.

The highest pH value was also observed at the combination of 5 t ha^{-1} of C and W i.e. C_5 W_5 at Epe. In the soil of Ijebu-waterside, the highest pH of 7.08 was observed at 5 t ha^{-1} of W application, i.e. C_0 W_5. This is in order because the more lime applied, the higher the pH as observed by some authors who reported that liming acid soil causes a significant increase in pH leading to a change in microbial activity (Baath *et al.,* 1980; Lang and Beese, 1985). Furthermore, at all levels of C application, the soil pH increased with the increasing rates of W application. This is due to the fact that cow dung as an organic manure also have the capacity to raise the soil pH because of its ability to provide more exchange sites for the basic cations in soil (Olayinka *et al.,* 1998).

Treatment effects on the nodulation and N-fixed by soybean

In the soil of Epe sown to un-inoculated soybean, the highest N-fixed observed at 5 t ha^{-1} each of both C and W applications (Fig. 7) could be attributed to the fact that the soil condition at this application rates was made conducive (pH near neutral) for the activities of the indigenous rhizobia. This was consistent with the works of some authors (Olayinka *et al.,* 1998; Adeboyo, 1985; Fried *et al.,* 1983). Olayinka *et al.* (1998) had earlier found that organic amendments could benefit rhizobia which are heterotrophs, and N_2 fixation, especially in soils low in indigenous organic matter. Adebayo (1985) also reported that cowpea rhizobium benefited from the additions of small amounts of manure.

Inoculation was also found to greatly increase the number of nodules and N-fixed. The increases in the number of nodules of the nodules of the inoculated over the un-inoculated soybean in the plot which produced the highest number of nodules at Epe and Ijebu-waterside were 76% and 98% respectively. The increase in N-fixed by the inoculated and the un-inoculated soybean over the control were 9 and 22% respectively, in the greenhouse. In the field, the N-fixed was also higher in soil of Ijebu-waterside than in Epe. The increase in N-fixed by the inoculated soybean in the field over the control was 39%.

In the inoculated pots, the highest N-fixed was also obtained at C_5 W_5 in both soils of Epe and Ijebu-waterside. This followed similar pattern observed in the un-inoculated plots. The general increase observed in N-fixed with respect to the increasing rate of W application especially at 5 and 10 t ha^{-1} C applications could also be attributed to the conducive environment created for the N_2 fixing rhizobia by the amelioratin of the soil pH through the W application. The N fixed by the inoculated soybean sown in Epe ranged from 55.65 to 58.65% while that of Ijebu-waterside ranged from 43.15 to 51.95%. This is in agreement with Herridge *et al.* (1990) who had earlier found that ureide N ranged from 15 to 60% in the field.

Although the grain yield was highest at C_0 $W_{2.5}$, it was not significantly different from that at C_5 W_5, which produced plants with the highest fixed N. In the soil of Ijebu-waterside, the N-fixed by the un-inoculated soybean was although highest at C_0 W_5, it was however, not significantly different from C_5 W_5 which was next to it in value. This confirms the finding that small amounts of manure benefits the rhizobia which are the N-fixers. The lowest values of N-fixed observed at the varying combinations of cow dung with zero rates of wood ash confirmed the importance of lime addition to acid soil to create conducive environment for microbial activities, which enhance N-fixation in legumes (Olayinka, 1990).

Combined treatment effects on the grain yield of soybean in the greenhouse

In both soils, the grain yields were also highest around the 5 t ha^{-1} of both C and W applications i.e. C_5 W_5, most especially at Epe location with lower pH and fertility. Any other treatment combinations which appeared higher in grain yield were not significantly different from C_5 W_5. This confirms further the effectiveness of this treatment combination to enhance the availability of nutrients due to the improved microbial activities as earlier observed by some authors (Mai and Feildler, 1979, Baath *et al.,* 1980, Lang and Beese, 1985). The increase in the number of pods and grain yield could therefore be attributed to the improved soil pH and the availability of nutrients to the plant for improved growth and yield.

SUMMARY AND CONCLUSION

This study showed that the growth and dry grain yield of soybean in the acid soils were improved by the applications of cow dung and wood ash either singly or in combination. The grain yield increase over the control ranged between 67 and 112% in the soil of Ijebu-waterside, and 39 to 86% in the soil of Epe in the greenhouse. Hence, wood ash and cow dung could be used either singly or in combination as cheap and readily available liming and fertilizing agents in these soils. While the grain yield in the greenhouse was found to be highest at C_5 W_5 in the greenhouse, the highest grain yield was obtained at C_5 $W_{2.5}$ in the field, especially at Ijebu-waterside. The percent increase in grain yield from the inoculated and the un-unoculated plots compared with the control were 33 and 56 respectively.

The percent increase in the N-fixed by the inoculated over the un-inoculated soybean at Ijebu-waterside was about 11. When the grain yield from the inoculated plot was compared with the un-inoculated, increases of 4 and 16% were obtained at Epe and Ijebu-waterside, respectively.

Nitrogen fixed was found to be highest at 5 t ha^{-1} of both cow dung and wood ash particularly with soil from Epe in the greenhouse. This rate could be considered in enhancing nitrogen fixation by soybean grown in this location and others of similar soil type. The N-fixed by the inoculated soybean sown in Epe ranged from 55.65 to 58.65% while that of Ijebu-waterside ranged from 43.15 to 51.95%. The percent increase in N-fixed by the inoculated and the un-inoculated soybean over the control were 9 and 22, respectively, in the greenhouse. In the field, the N-fixed was higher in soil of Ijebu-waterside than in Epe. The increase in N-fixed by the inoculated soybean in the field over the control was 39 39%.

The treatment combination (5 t ha^{-1} each of both cow dung and wood ash) could therefore be considered when planting soybean in acid soils of similar characteristics for improved growth and grain yield.

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Table 1: Physico-chemical characteristics of the experimental soils and the amendments

Properties	Epe	Ijebu-waterside	Wood ash	Cow dung
pH (H_2O)	5.3	5.5	11.3	81
Sand (g/kg^{-1})	892	900		
Silt (g/kg^{-1})	42	40		
Clay (g/kg^{-1})	66	60		
Textural class	Loamy sand	Loamy sand		
Organic Carbon (g/kg^{-1})	5.4	20.8	8.8	44.3
Total Nitrogen (g/kg^{-1})	0.9	1.8	0.9	3.6
C:N	6.0	11.6	9.8	12.3
Available P (mg/kg^{-1})	13.2	7.99	26.98	85.32
Exchangeable acidity (cmol kg^{-1})	0.05	0.04		
Exchangeable Ca (cmol kg^{-1})	2.78	0.62	4.56	4.68
Mg (cmol kg^{-1})	1.21	0.31	2.83	2.92
K (cmol kg^{-1})	0.42	0.20	4.1	16
Na (cmol kg^{-1})	0.93	0.35	1.7	8.9

Table 2: Effect of single application of wood ash and cow dung on the pH of the soils obtained from Epe and Ijebu-waterside.

Amendments ($t\ ha^{-1}$)	pH (H_2O) EPE 4 WAP	EPE 8 WAP	IJEBU-WATERSIDE 4 WAP	IJEBU-WATERSIDE 8 WAP
Wood ash				
0	5.46c	6.08b	5.99c	6.27b
2.5	5.89b	6.44a	6.56b	6.56b
5.0	6.09a	6.50a	6.79a	6.81a
Cow dung				
0	5.81	6.31	6.47	6.66
5	5.93	6.34	6.53	6.62
10	5.87	6.37	6.35	6.56
	ns	na	na	na

Means with the same letters in columns are not significantly (P<0.05) different according to Duncan's Multiple Range Test.

Table 3: Effect of combined applications of cow dung and wood ash on the pH of the soils from Epe and Ijebu-waterside

Amendments ($t\ ha^{-1}$)	pH (H_2O) EPE 4 WAP	EPE 8 WAP	IJEBU-WATERSIDE 4 WAP	IJEBU-WATERSIDE 8 WAP
$C_0\ W_0$	5.52de	6.27ab	5.98c	6.49cd
$C_5\ W_0$	5.46e	5.98b	6.03c	6.19e
$C_{10}\ W_0$	5.71cde	6.27ab	5.97c	6.34de
$C_0\ W_{2.5}$	5.86bcd	6.42ab	6.69ab	6.87ab
$C_5\ W_{2.5}$	6.02abc	6.51a	6.72ab	6.85b
$C_{10}\ W_{2.5}$	5.93abc	6.41ab	6.38bc	6.70bc
$C_0\ W_5$	6.27a	6.61a	6.86a	7.08a
$C_5\ W_5$	6.27a	6.40ab	6.81ab	6.87ab
$C_{10}\ W_5$	6.12ab	6.58a	6.85a	6.57cd

Means with the same letters in columns are not significantly (P<0.05) different according to Duncan's Multiple Range Test; C = Cow dung; W = Wood ash; 0, 2.5, 5.0, and 10 rates of amendments $t\ ha^{-1}$.

Table 4: Soil pH of Epe and Ijebu-Waterside fields as affected by single application of cow dung and wood ash under field conditions

Amendments ($t\ ha^{-1}$)	Soil pH Epe	Soil pH Ijebu-Waterside
C_0	6.37	6.33a
C_5	6.08ab	6.41a
C_{10}	6.18b	6.30a
W_0	5.99b	6.15b
$W_{2.5}$	6.29a	6.42a
$W_{5.0}$	6.35a	6.46a

Means with the same letters in columns are not significantly (P<0.05) different according to Duncan's Multiple Range Test; C = Cow dung; W = Wood ash; 0, 2.5, 5.0, and 10 rates of amendments $t\ ha^{-1}$.

Table 5: Effect of cow dung and wood ash on N-fixed by the inoculated and un-inoculated soybean at 8 WAP under field conditions at Epe and Ijebu-waterside

Amendments ($t\ ha^{-1}$)	N-fixed (inoculated)		N-fixed (un-inoculated)	
	Epe	Ijebu	Epe	Ijebu
$C_0\ W_0$	53.16	41.94b	47.81	47.38b
$C_5\ W_0$	45.54	55.13a	50.29	56.55ab
$C_{10}\ W_0$	50.20	53.22ab	47.38	50.66ab
$C_0\ W_{2.5}$	50.18	56.39a	49.14	60.72a
$C_5\ W_{2.5}$	45.58	55.04a	47.82	54.79ab
$C_{10}\ W_{2.5}$	52.72	52.95ab	52.42	57.27ab
$C_0\ W_5$	49.00	51.84ab	49.28	53.93ab
$C_5\ W_5$	49.89	55.04a	51.72	56.66ab
$C_{10}\ W_5$	52.11	58.15a	50.55	52.27ab
	na		na	

Means with the same letters in columns are not significantly ($P<0.05$) different according to Duncan's Multiple Range Test; C = Cow dung; W = Wood ash; 0, 2.5, 5.0, and 10 rates of amendments $t\ ha^{-1}$.

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