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Editorial message

On behalf of the Editorial Board of the Journal of Agriculture & Forestry Research (JAFR) and myself, it is a pleasure to publish the second issue of Volume 2. The process of improving the quality of a scientific journal is ongoing. We are dedicated to this process. Journal of Agriculture & Forestry Research (JAFR) is an open-access multidisciplinary journal that publishes fundamental and applied research works in different areas of Agriculture and Forestry. JAFR publishes papers concerned with the advance of agriculture and the use of land resources throughout the world.

I would like to acknowledge our users, contributors, authors, and reviewers for volunteering to help the journal's success and development mission. We publish our publications with an emphasis on quality, safety, and improved research. We hope that JAFR will be an enlightening and inspirational platform for both researchers and users, laying the path for a groundbreaking future.

Editor-in-chief
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Research Article

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Haematological and Biochemical Profiles of Exotic and Nigerian Locally Adapted Turkeys Reared in a Tropical Environment

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ABSTRACT

Variations in haematological and biochemical parameters of birds can be used to explain differences in the adaptability and response of different breeds to the local environment. The study aimed at determining the haematological and biochemical variation of Nigerian locally adapted (with different plumage colours) and exotic turkeys reared in tropical environments. A total of 200 day-old poults comprising 150 local (50 Black, 50 Lavender and 50 White) and 50 exotic (Nicholas White) turkeys were used in this study. Blood samples were collected from 180(45 from each genotype) turkeys for the estimation of haematological and biochemical parameters. Genotypes were observed to have a significant ($p<0.05$) effect on the haematological and biochemical parameters. The local white turkey had the highest ($p<0.05$) white blood cell (WBC) (53.22) and monocytes (MONO) while red blood cell (RBC) was highest ($p<0.05$) in local black but similar ($p>0.05$) to the values in local white and exotic turkeys. The mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and lymphocyte (LYMP) were highest ($p<0.05$) in local lavender but similar ($p>0.05$) to the values in local white and exotic turkeys. The local white turkey had the highest ($p<0.05$) value of urea, Creatinine and Alanine aminotransferase (ALT) while the Cholesterol level was highest ($p<0.05$) in the local black turkey. The exotic turkey had the highest ($p<0.05$) levels of total protein, globulin, glucose and aspartate aminotransferase (AST) while the albumin level was highest ($p<0.05$) in the local white. There are genotypic variations in haematological and serum biochemical of turkeys examined in this study. Therefore, the haematological performances observed in these turkeys can be used as a reference in case of any deviation during disease conditions and also in the development of an adapted turkey line for this environment.

INTRODUCTION

Turkeys are classed in the family of Phasianidae in the taxonomic order of Galliformes (Crowe et al. 2006). The origin is somewhat controversial, however, it was reported that the archaeological specimen of wild turkey was found in North America that date to the Pleistocene and turkeys were symbolic of many indigenous groups in North America (Thornton and Emery, 2017) while another view affirmed that the turkey raised in central Mexico was ancestral of all the varieties of domestic turkeys we have today (Dominguez-May et al. 2021). Turkey has gained global recognition due to the high demand for its product (meat) (Yakubu et al. 2013).

The local turkeys found in Nigeria are hardy and often show greater resistance to most tropical poultry diseases. However, they are characterized by small body weight and low production (Ngu et al. 2014). On the other hand, the exotic turkeys had been selected over generations for early maturity and high production of meat (Ilori et al. 2011) but they relatively have low immunity compared to the tropically adapted breeds (Oguntade et al.2021). The immune and health status of animals can greatly influence their productivity and these can be assessed through haematological and biochemical analyses (Abdi-Hachesoo et al. 2013). In addition, blood parameters such as packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) will give the idea of feed utilization, cellular respiration and immune condition in animals (Kral and Suchy, 2000).

Evaluation of the haemato-biochemical parameters in birds can be used to determine a response to its internal and external environment (Esonu et al. 2001). The health and functional status of vital body organs (such as the pancreas, heart, muscles, liver and kidney) of an animal can be ascertained through the assessment of plasma or sera components (Agina et al. 2015). Hence, haematological and biochemical analyses could be used as diagnostic tools to monitor the effectiveness of recommended and applied treatment including determining the toxicity of drugs and chemical substances and forming a prognosis (Alimi et al. 2020) that will curb the future occurrence of ill health. Although, the haematological and serum biochemical parameters of animals are influenced by prevailing environmental and physiological conditions of animals (NseAbasi et al. 2014). Nevertheless, great variability can be adduced to genotypic differences. Hence, this study would add to the previous knowledge (Ilori et al. 2011; Isidahomen et al. 2013; Ajaonuma et al. 2013; Oguntade et al. 2021) on genetic variation in haematology and biochemistry of tropically adapted and exotic turkeys, which will provide information for the development of tropically adapted broiler turkeys. Therefore, this study assessed the hematological and biochemical profiles of exotic and Nigerian locally adapted turkeys reared in a tropical environment.

MATERIALS AND METHODS

Description of Experimental Site

This research was done at the Turkey Breeding unit of the Teaching and Research Farm of Ambrose Alli University, Ekpoma Edo State, Nigeria. The study which lasted for twenty weeks was carried out between January and June, 2020.

Experimental Birds and Management

A total of two hundred (200) turkeys made up of one hundred and fifty (150) Nigerian locally adapted (50 each of black, lavender and white genotypes) and fifty (50) Nicholas white (exotic) day-old poults were used for this study. The two breeds were sourced from a reputable hatchery in Ibadan, Oyo state Nigeria. The birds were raised in deep litter pens. They were brooded for a period of four weeks with strict adherence to brooding management practices. The poults were vaccinated against Marek's disease, Newcastle disease and infectious bronchitis at day old from the hatchery. Subsequent vaccinations and medication were provided routinely and as required. The birds were fed commercially available diets ad libitum and provided constant access to clean water. All the experimental birds were identified individually through a labelled tag attached to their wings. All the experimental turkeys were maintained under the same experimental management conditions.

Data Collection

Blood samples (about 2.5 ml) were collected from the superficial ulnar vein of 180 turkeys (45 from each genotypic group) at the end of the experiment. About 1 ml of the blood collected was transferred into a tube containing EDTA (ethylene diaminetetracetic acid) for haematological analysis and the other part of the blood was transferred into the plain bottle for biochemical analysis. The routine haematological procedures for avian16 were used to determine the packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and differential leukocyte counts. An automated cell counter was used to measure the RBC, haemoglobin (Hb) and WBC within 24 hours after the collection of blood16. The cyanomethemoglobin method was used to measure the Hb concentration while the PCV was measured by the michrohematocrit method in capillary tubes, centrifuged at 12,000g for 5 minutes (Samour et al. 2010). Other haematological indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated (Samour et al. 2010). Total protein was determined using the direct biuret method, while the bromocresol green method was used for the determination of serum albumin (Fafiolu and Alabi, 2020). Serum globulin was calculated by subtracting albumin from total protein. Creatinine and urea were determined (Fafiolu and Alabi, 2020). The serum cholesterol and alanine amino transferase (AST and ALT) parameters as well, as alkaline phosphate (ALP) were determined as described by Samour et al. (2010)

Statistical analysis

The data collected were analyzed using the General Linear Model (GLM) procedure of SAS (2014) using the model below:

Yijk = μ + Gi + εij

Where:

Yijk = the dependent variable (haematological and biochemical parameters).

μ = Overall population mean

Gi = Effect of the ith turkey genotypes

εij = The residual random error.

The mean separation was done using Tukey's HSD of the same software

RESULTS

Effect of genotype on hematological parameters of Nigerian local and exotic turkeys

Table 1 showed the effect of genotype on turkey haematological parameters of different plumage colours of Nigerian local and exotic turkeys. Genotype had a significant ($p<0.05$) effect on all the haematological parameters except Packed Cell Volume (PCV), Hemoglobin (Hb), Mean cell Haemoglobin concentration (MCHC), Platelet (PLAT) and Neutrophil (NEU). Genotype had a significant effect ($p<0.05$) on white blood cells (WBC) with the highest level of WBC recorded in local white turkey (53.22 103µl-1) which is not significantly ($p>0.05$) different from that of local black turkey (47.96103µl-1)and exotic turkey (50.12 103µl-1). The lowest level of WBC was however recorded in local lavender turkey (45.82 103µl-1) which was comparable ($p>0.05$) with the values recorded in local black and exotic turkey. The highest ($p<0.05$) level of RBC was recorded in local white (3.40 106µl-1) and black (3.19 106µl-1) including exotic turkey (3.18 106µl-1) while the least was obtained in the local lavender turkey (2.70 106µl-1). Conversely, the local lavender had the highest ($p<0.05$) values of mean corpuscular haemoglobin (MCH) (54.07 pg/cell), mean corpuscular volume (MCV) (150fL) and lymphocyte (LYMP) (85.62 %). Local black turkey had the least level of MCH (51.6 pg/cell) and MCV (141.68 fL).The highest level of lymphocyte observed in lavender local turkey was not significantly ($p>0.05$) different from the values observed in local black (84.16 %) and white turkey (84.37 %) but significantly ($p<0.05$) different from the level observed in exotic turkey (82.33 %).

Further, the percentage of monocyte was highest ($p<0.05$) in local white (8.78 %) turkey which was not significantly ($p>0.05$) different from the level observed in local black turkey (8.28 %) followed by 7.77 % observed in exotic turkey. The local lavender turkey however had the lowest percentage of monocyte (6.58 %).

Table 1: Effect of genotype on haematological parameters of different plumage colours of Nigerian local and exotic turkey

Parameters	Local black turkey	Local lavender turkey	Local white turkey	Exotic turkey
WBC(10^3.µl^{-1})	47.96±1.53^{ab}	45.82±1.29^{b}	53.22±1.62^{a}	50.12±2.70^{ab}
RBC(10^6µl^{-1})	3.19±0.11^{a}	2.70±0.07^{b}	3.40±0.13^{a}	3.18±0.22^{a}
PCV (%)	41.95±0.81	41.67±1.14	45.47±0.60	41.45±2.05^{NS}
MCH(pg/cell)	51.60±0.43^{b}	54.07±0.46^{a}	53.82±0.44^{a}	53.45±0.41^{a}
HB(gdl^{-1})	16.52±0.65	15.05±0.54	16.93±0.32	15.42±0.81
MCHC(g/dl)	36.60±0.33	36.93±0.27	36.72±0.73	36.76±0.52
MCV(fL)	141.68±1.59^{c}	150.00±0.81^{a}	144.73±0.62^{bc}	146.70±1.36^{ab}
LYMP (%)	84.16±0.58^{ab}	85.62±0.87^{a}	84.37±1.26^{ab}	82.33±0.80^{b}
PLAT (%)	89.33±1.69^{a}	135.50±14.16^{a}	137.67±26.87^{a}	108.83±10.80^{a}
NEU (%)	8.93±0.15^{a}	8.82±0.51^{a}	9.53±0.68^{a}	10.42±0.66^{a}
MONO (%)	8.28±0.15^{ab}	6.58±0.10^{c}	8.78±0.34^{a}	7.77±0.05^{b}

Note: abc Means in the same column in the same group with the different superscripts are significantly different (P<0.05)

WBC: White blood cell; RBC: Red blood cell; PCV: Packed cell volume; MCH: Mean cell Haemoglobin; Hb = Haemoglobin Concentration; MCHC: Mean cell Haemoglobin concentration; MCV: Mean cell volume; LYMP: Lymphocyte; PLAT: Platelet; NEU: Neutrophi; MONO: Monocyte.

Effect of genotype on Serum Biochemistry and lipid profile of Nigerian local and exotic turkeys

Table 2 showed the effect of genotype on turkey serum biochemistry and lipid profile. Genotype had a significant effect on all the serum biochemistry and lipid profile except the level of alkaline phosphatase (ALP). Genotype significantly ($p<0.05$) affect the level of urea discovered in the turkey used for this study. The lowest level of urea was discovered in black local turkey (4.33 mg/dl) while the highest level was discovered in local white (6.80 mg/dl) but similar to the values obtained in lavender (6.17 mg/dl) and exotic (6.16mg/dl) turkeys. Creatinine level was highest ($p<0.05$) in local white (0.77mg/dl) but comparable with the value in exotic turkey (0.77mg/dl) while local lavender turkey had the least value (0.63 mg/dl) of creatinine. The level of cholesterol was also significantly ($p<0.05$) affected by genotype. The highest level of this lipid was detected in local black (96.83mmol/l) which was not different ($p>0.05$) from the values in local white (95.00mmol/l)and exotic turkeys (94.83mmol/l) while the lowest level of cholesterol was discovered in local lavender turkey (83.67mmol/l).The total protein value in these turkeys was significantly ($p<0.05$) highest in exotic turkey (5.73g/l) followed by that of local white turkey (4.87 g/l)and black (4.53 g/l) while the least (4.28 g/l) was observed in the local lavender turkey. Albumin level was also significantly ($p<0.05$) affected by genotype. The local black turkey had the least (2.30g/l) while the highest level of albumin was observed in the exotic turkey (2.70 g/l) with similar ($p>0.05$) values in local white (2.60 g/l) and lavender (2.55 g/l) turkeys. The level of globulin in the turkeys used in this study followed similar trend as the values obtained in total protein. In addition, the glucose level was significantly ($p<0.05$) highest in exotic turkey (133.83mg/dl) but was not significantly ($p>0.05$) different from the value obtained in local black (127.16 mg/dl) while the least was discovered in local lavender turkey (123.33 mg/dl) which was also similar ($p>0.05$) to that of local white turkey (118.17 mg/dl).

The highest ($p<0.05$) level of alanine aminotransferase (ALT) level was detected in local white turkey (15.33IU/L) while the least was observed in exotic turkey (6.00IU/L) comparable with the values in local black (6.33 IU/L) and local lavender (7.83 IU/L).Aspartate aminotransferase (AST) level was significantly ($p<0.05$) affected by genotype. The highest level of AST was detected in exotic turkey (171.00 IU/L) while the least was observed in local lavender (133.00 IU/L), local black turkey (143.83 IU/L) and local white (144.50IU/L).

Table 2: Effect of genotype on serum biochemistry and lipid profileof different plumage colours of local and exotic turkey

Parameters	Local black turkey	Local lavender turkey	Local white turkey	Exotic turkey
UREA	4.33 ± 0.21^{b}	6.17 ± 0.40^{a}	6.80 ± 0.2^{a}	$6.16\pm.40^{a}$
CREAT	0.72 ± 0.04^{ab}	0.63 ± 0.04^{b}	0.77 ± 0.04^{a}	0.77 ± 0.04^{a}
CHOL	96.83 ± 2.55^{a}	83.67 ± 2.09^{b}	95.00 ± 3.33^{a}	94.83 ± 1.28^{a}
TP	4.53 ± 0.10^{b}	4.28 ± 0.15^{c}	4.87 ± 0.06^{b}	5.73 ± 0.11^{a}
ALB	2.30 ± 0.03^{b}	2.55 ± 0.07^{a}	2.6 ± 0.06^{a}	2.70 ± 0.08^{a}
GLOUB	2.23 ± 0.04^{b}	1.73 ± 0.06^{c}	2.27 ± 0.08^{b}	3.03 ± 0.09^{a}
GLUCO	127.16 ± 4.2^{ab}	123.33 ± 1.31^{bc}	118.17 ± 1.11^{c}	133.83 ± 2.01^{a}
ALT	6.33 ± 0.49^{b}	7.83 ± 0.75^{b}	15.33 ± 2.60^{a}	6.00 ± 0.63^{b}
AST	143.83 ± 4.64^{b}	133.00 ± 2.05^{b}	144.50 ± 3.07^{b}	171.00 ± 5.77^{a}

Note: abc Means in the same column in the same group with the different superscripts are significantly different ($P<0.05$)

Urea (mg/dl); CREAT: Creatinine (mg/dl); CHOL: Cholesterol (mmol/l); TP: Total protein (g/l); ALB: Albumin (g/l); GLOUB: Globulin (g/l); GLUCO: Glucose (mg/dl); ALT: Alanine aminotransferase (IU/L); AST: Aspartate aminotransferase (IU/L); ALP: Alkaline phosphatase (IU/L)

DISCUSSION

The result of the haematological profile of local and exotic turkey genotypes in the current study showed that the haematological parameters of turkeys were influenced by the genotypes and feather colour of Nigerian indigenous turkeys. The highest level of WBC was recorded in local white turkey which is not significantly different from that of local black turkey and exotic turkey but lowest in lavender turkey. This could suggest the better genetic potential of local white turkeys to resist infectious diseases in the tropical climate of Nigeria as they have been naturally selected for

adaptation in this environment rather than production. The major functions of the WBC and its differentials are to fight infections and protect the body against foreign organisms by generating antibodies in immune response (NseAbasi et al. 2014). In addition, animals with low WBCs are exposed to a high risk of disease infection, while those with high WBCs are capable of producing antibodies to resist invasion by pathogens and thereby adapt better to local and disease prevalent environments (NseAbasi et al. 2014). Thus, the local white turkey is superior in terms of survival to the disease prevalent environment. Generally, the values of WBC obtained for all the turkey genotypes in this study, are in the range of the values reported by Isidahomen et al. (2013) but lower than the values reported by Odunitan-Wayas et al. (2017) for male and female turkeys. The highest level of RBC was recorded in local black and white including exotic turkey while the least was however recorded in the local lavender turkey. The values of RBC obtained for all the turkey genotypes in the current study fell within the physiological range (2.28—2.81) reported by Daniel-Igwe and Okwara (2018) on turkey. Since RBC helps mainly to take oxygen (through haemoglobin) from the lungs to tissues and removes carbon dioxide out of the body tissues through the lungs (NseAbasi et al. 2014). Therefore, the RBC values in this study implies better chances of healthy living and interaction with the environment in all the turkey genotypes and also signifies that the exotic turkey used in the study are locally adapted to the environment. Moreover, there observed no significant difference in the level of PCV in the study populations. However, the value of PCV obtained for both local and exotic turkeys were within the range of values (39.77±0.46) for matured turkey as reported by Priya and Gomathy (2008) and (30.66±0.91) reported by Pandian et al. (2012) on Indian turkey. Furthermore, since the PCV is associated with nutritional status (Isidahomen et al. 2013), it is therefore suggested that the values of PCV obtained in the current study for all the turkey genotypes indicate that they were healthy; showed better feed utilization and hence better adaptation to the rearing environment. In addition, the Hb and MCHC were not significantly influenced by genotype and their values are within the normal range reported by several authors (Bounous and Stedman, 2000). The level of MCH, MCV, LYMP and Mono were significantly influenced by genotype and plumage colour. The MCV shows the average erythrocyte size while the MCH indicates the haemoglobin amount per blood cell (Bounous and Stedman, 2000). The higher level of the two indices in both local and exotic turkeys except in some feather colour in the Nigerian local turkey suggested that there is no reduction in cellular oxygen requirements in order to adjust to environmental stressors such as anemic disease condition (NseAbasi et al. 2014).

Further, the percentage LYMP was influenced by genotypes with the highest values observed in local turkeys. LYMP changes in concentration in an individual may suggest an interaction between the pathogen and the host. The B lymphocytes are responsible for the rapid generation of antibodies specific to a particular pathogen while T lymphocytes regulate lymphocyte functions and acute viral infection (Pei et al. 2003).In addition, natural variability (Fair et al. 2008) and immune-competence (Berndt and Methner, 2001;Juul-Madsen et al. 2006) were reported in studies of subpopulations of lymphocytes in avian blood. The values of LYMP in this study reveals immune variability among the four turkey populations while the high level of LYMP in local turkey especially in lavender local turkey suggests that local turkey are much more immune-competent in response to viral infection in the tropical climate when compared to their exotic counterpart. Moreover, monocytes (MONO) are the biggest type of white blood cell and function in the fight against bacteria, viruses and fungi in the body. Their function primarily is to ward off diseases and infections. These monocytes migrate from the peripheral blood to the tissues and differentiate into macrophages whose morphology and function are dependent on the organ and tissue in which they are present (Geissmann et al. 2010). The level of MONO reported in our work is higher than the level reported by Schmidt et al. (2009) in Bronze turkey which suggests that the turkey used in this study is well adapted to the tropical environment and is able to mount enough immune response against infections that could be brought about by any class of pathogen.

The variation in the levels of urea in the blood of the turkeys with the least value in the black local turkey suggests differences in renal functions associated with metabolic activities in different genotypes of the turkey breeds. Interestingly, creatinine was high in exotic and local white turkey which was not significantly different from those of local black turkey but low in lavender local turkey. Creatinine is the final metabolite of creatine conversion and a major marker of kidney function. Our results may indicate the better conversion of phosphocreatine to creatinine in the muscle, which suggest reduction in the use of phosphocreatine for muscle contraction. It should be noted that normal functioning of the kidney will result in rapid excretion of Creatinine (Mathuria and Verma, 2008). Increased urea and creatinine as indices of impaired kidney function in aflatoxicosis were reported in chickens and rats (Hassan et al. 2012). However, the levels observed in this study

are within the range reported in poultry (Naseem et al. 2018).

Cholesterol was significantly influenced by the genotype of the turkey. The higher level of cholesterol was observed in exotic, local black and local white turkeys while the lavender had the least level of cholesterol. The highly similar pattern of the measured serum cholesterol may be attributed to the possibility of increased metabolism which often influences cholesterol concentration in avian blood serum. The significantly high level of total protein in the exotic turkey compared to the different indigenous turkeys of different feather colours may be due to improved growth rate coupled with more muscle to the bone ratio in the turkey compared to the indigenous turkey, which is having better ability to convert the feed to muscle than the indigenous turkey. Sera total proteins are currently being used as a diagnostic parameter to ascertain the health condition in birds and together with albumin can give the protein synthesis (Piotrowska et al. 2011). Albumin is high in the turkeys used except in local black turkeys with a lower level. The values of albumin concentration obtained in this study followed the TP concentration. This suggests the ability of both turkey genotypes to utilize the total available protein from the diets. The level of serum globulin observed with the exotic turkey having the highest level followed by the local black, local white with the least observed in the local lavender turkey may be indicative of an enhanced immune system especially in the exotic turkey as the concentration of serum albumin proteins antioxidant status are regarded as the direct reference to the body immune function (Zhang et al. 2013).

Glucose forms the major source of energy for cellular metabolism. In the current study, the exotic and local black turkeys had higher blood glucose. This slight increase indirectly suggests that these groups possess better absorption of nutrients and enhanced liver glycogenesis (Kokore et al. 2021).

In this study, the serum AST, ALT and ALP differ significantly with turkey genotypes. Higher level of ALP was detected in exotic and local black turkeys. An increase in serum ALP has been attributed to rapid growth and bone activity as reported by Lowe et al. (2022). This is not surprising as the exotic turkey has the highest rate of growth because of its selection for optimum growth when compared with the local counterpart (Ilori et al. 2011).The mean values of ALT and AST are in accordance with the other researchers (Bounous et al. 2000; Ibrahim et al. 2012).

Generally, analysis of blood parameters would provide a reliable indicator of health status of an animal. The values of some blood biochemical parameters namely the glucose, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and urea; could form a diagnostic tool to confirm various disease conditions and unravel the abnormal function of vital body organs such as liver disorder, kidney disease, diarrhoea and dehydration (Akporhuarho, 2011). Therefore, information on the blood profiles of various breeds of turkeys could form useful diagnostic tools which would further enhance the development of improved tropical turkey breeds. Although several authors have reported values for the various haematological and serum biochemical parameters of turkey (Patra et al. 2008; Ibrahim et al. 2012), however, there is a need for adequate information on the variability of these values in the different plumage colours as we have in the tropical region such as Nigeria.

CONCLUSION

The current study established genetic variability in haematological and biochemical parameters of Nigerian locally adapted and exotic turkeys. The Nigerian locally adapted turkeys also displayed variations along the three plumage colours. Generally, through haematological and biochemical analysis, it can be deduced from the current study that, the local turkey has a better chance to adapt and survive while the exotic turkey as well is locally adapted to the harsh and disease-prevalent environment of the tropical climate in the study area.

Significance statement

The study discovered that the turkey genotype had a significant effect on all the haematological parameters except Packed Cell Volume (PCV), Hemoglobin (Hb), Mean cell Haemoglobin concentration (MCHC), Platelet (PLAT) and Neutrophil (NEU).

Also, the serum biochemical and lipid parameters examined were significantly affected by genotype, except for the level of alkaline phosphatase (ALP).

Most of the information available on turkey haematology and serum biochemistry was not considered a variation of the values based on plumage colour. Therefore, the variability in haematological and serum biochemical parameters observed among different plumage colours of Nigerian locally adapted turkeys and exotic turkeys would form the basis for screening of Nigerian locally adapted turkeys into different immune-competent groups based on the values of their haematological and

biochemical parameters and thus help in the development of tropical broiler-type turkey.

ETHICAL APPROVAL

Ethical clearance was given by the Federal University of Agriculture Abeokuta Ogun State (FUNAAB), Nigeria ethical review board in accordance with international standards on the care and use of experimental animals.

Conflict of interest

The authors declared no conflict of interest.

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Research Article

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Cluster-Based Demonstration and Popularization of Highland Maize (BH661) and Midland Maize (BH547) Production Technologies Packages in Selected Districts of Gedeo Zone and Sidama Region, Ethiopia

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ABSTRACT

Improved maize varieties, pesticides, lime, NPSB, and urea fertilizers, as well as suggested agronomic procedures, were used as inputs for the display. Sites were selected based on the potentiality of the area to produce maize as well as their accessibility by land and road. Farmers, development agents, experts, and other stakeholders received various forms of training before implementing the demonstration. Field visits, field days, and yield harvesting events are all included as evaluation means of the demonstration. The findings show that the Gedeb and Windogenet areas produced a mean grain yield of maize of 42 qt/ha-1 and 44 qt/ha-1, respectively. The grain yield, the number of cobs per plant, disease resistance, seed color, and the well-covered seed of the varieties were preferred by the producers. The encountered challenges were the occurrence of fall armyworm; however, the worm was controlled by applying chemicals. Also, the lesson obtained from the demonstration was appropriately applying recommended maize packages is the major solution to the production and productivity problem of farmers. Extension personnel and concerned bodies need to work on the appropriate application of recommended maize technologies to improve maize productivity of farmers and interested bodywork on maize production.

INTRODUCTION

Ethiopia is the fifth largest producer of maize in Africa and smallholder farmers make up 94 % of the crop production. The country produces white maize, the preferred type of maize in neighboring markets. As the cheapest source of caloric intake in Ethiopia, providing 16.7 % of per capita calorie intake nationally, maize is an important crop for overall food security (CSA, 2015).

Maize is everything for Ethiopian maize farmers. Three fourth of the maize produced is consumed at the household level by the small-scale producers themselves (CSA, 2017). The grain is consumed in different forms of food; the Stover is used as feed, fuel, and construction material. Besides, it serves as a major source of income and means of employment for tens of millions of farming and business communities. Due to its wider significance in the country, maize is one of the strategic field crops targeted to ensure food security in Ethiopia (Keno et al. 2018).

Despite the importance of maize as a principal food crop, its average yield in Ethiopia (3.6-tons ha-1) is still lower than that of the world's average (5.6 t ha-1 in 2016) (FAO, 2017). A significant portion of this yield gap is attributable to biotic and abiotic stresses. The low productivity of maize is attributed to many factors like the frequent occurrence of drought, decline of soil fertility, poor agronomic practice, cease/limited use of fertilizer, insufficient technology generation, and adoption, lack of credit facilities, poor seed quality, disease, insect, pests,

and weeds (Dhliwayo, et al. 2009). Weak research-extension linkage is also a major bottleneck for the low awareness and adoption of improved agricultural technologies. For that creating various initiatives to strengthen the research-extension-farmer linkage is an important mechanism to be able to bridge the gap and on-farm demonstration of improved maize variety with associative inputs, including farmers' pieces of training are important to facilitate change in the behavior of farmers and ultimately bring behavioral changes in favor of improved maize technology adoption and extension package (Dawit et al. 2014). Therefore, this cluster-based large-scale demonstration was conducted to improve maize productivity by creating awareness of the appropriate application of recommended maize packages and to evaluate farmers' feedback on technology thereby ultimately enhancing maize productivity improvement of smallholder farmers.

METHODOLOGIES AND USED APPROACHES

Before beginning the demonstration tasks, extension staff and relevant administrative bodies at the zone and district levels held all required discussions and communications regarding the goals and significance of carrying out the activity. After that, the district and kebele were purposefully chosen based on how well the region suited the technology (production potential and accessibility). Additionally, the farmers were chosen in consultation with district experts and development agents, taking cluster-based demonstration principles into account. The amount of area coverage that was intended to be implemented restricted the number of host farmers; therefore, the most important factor was adjacent farmland, up to the achievements of the planned hectare of land.

Training

At the starting point of implementation of the demonstration, training was given to selected farmers, DAs, and experts from the woreda farm and natural resource development office on agronomic practices, objectives, and the importance of a cluster-based demonstration approach.

All necessary inputs were collected through the collaborative contributions of both HwARC and Weareda Farm and the Natural Resource Development Office. Indeed, improved maize seed (5 quintals), fertilizers (40 quintals of NPSB and 30 quintals of urea), awareness-creation and capacity-building training, and field day ceremonies were prepared by the research center. Additionally, soil lime and chemicals have been collected under the responsibility of the woreda farm and natural resource development office. Then input distribution was done by considering the selected land size for a demonstration from each beneficiary farmer, which was accomplished by the collaborative responsibility of Kebele Das coordinators, the kebele chief adumbrative or chairman, experts from the woreda farm and natural resource development office, and respective researchers from the research center.

All necessary agronomic practices were done carefully, starting from land preparation up to yield harvesting, by applying the joint responsibility of beneficiary farmers, kebele and woreda agricultural officers, and researchers playing their respective roles. Three times (once before sowing plus once during sowing), the farming frequency was done: at the time of sowing 5 quintals ha-1, lime was applied by dressing in a row; 100 percent NPSB and 25 percent urea were applied at the sowing session; and the remaining 75 percent urea was applied 35 to 40 days after sowing as part of integrative pest and insect management (chemical and biological insect management practices were applied to control the pole worm).

Integrative continuous follow-up (inclusive of researcher-farmers-extension) was done periodically by strengthening good practices and taking corrective measures for miss field management practices by visiting each host farmer's field and having discussions, and by recommending making a frequent visit to each host farmer alone his or her demonstration field to make communication with DAs. Each demonstration task was performed by applying a participatory and shared responsibility approach, starting from the planning phase to the end, which was done by making effective communication with all stakeholders at each stage (researchers, extension personnel, administrative staff, and host farmers). This approach was done by sharing input costs, taking common field management measures, and following up by participating in multidisciplinary research teams.

ACHIEVEMENTS

Field day

At both locations (Gedeb and Wondo genet) field day was conducted with inclusive participation of all stakeholders (zone, woreda, and kebele extension personals, farmers, SARI, and HwARC researchers and management

members). On-field day, media (southern radio and television) coverage was employed.

Yield performance

Two demonstrated maize varieties (BH547 and BH661) exhibited better yield performance in their respective demonstrated locations, as shown in the grain yield performance table 3. This improved yield performance was brought about by the implementation of full packages. This indicates that the main productivity potential barrier to the maize in the Gedeo and Sidama areas is the failure to implement the recommendations fully.

Table 1. Participant list of training

Location	Participant list in training												Grand Total
	Farmer			Agri- expert			Researcher			Other officers			
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	
Wondo genet	15	5	20	6	2	8	6	2	8	7	2	9	45
Gedeb	10	5	15	6	1	7	6	2	8	7	2	9	39
Sub-total	25	10	35	12	1	15	12	4	16	14	4	18	84

Source: field data, 2022

Table 2. Participant list in field day

Location	Participant lists												Total
	Farmers			Agri-experts			Researchers			Other officers			
	male	female	total	male	female	total	male	female	total	male	female	total	
Wondo genet	57	17	74	9	2	11	7	2	9	12	2	14	**107**
Gedeb	111	16	127	12	2	14	6	2	8	16	5	21	**170**
Sub-total	168	33	201	21	4	25	13	4	17	28	7	35	**277**

Source; field data, 2022

Fig-1 Field day photo at Wondogenet and Gedeb districts, 2022

Table 3. Yield performance

District	Kebele	variety	Grain yield in quintal per hector		
			min	max	Mean
Wondo genet	Yuwe (N=12)	BH547	39.3	47	**43**
	Aroma (N=8)	BH547	40	48.6	**44.3**
Gedeb	Galcha(N =17)	BH661	39	47	**42.5**
	Gubata(N=12)	BH661	38.3	45.6	42

Source: field data, 2022

Feedbacks given

Farmers expressed that this comprehensive demonstration of the technologies and applied approaches was practically approved as a means of increasing maize production and productivity. Indeed, the majority of the maize plants on the demonstration site had two to three cobs per plant, whereas the same maize variety planted in neighboring farmers' fields and outside the demonstration field had just one cob per plant. Operationalizing the complete packages is what accounts for this productivity disparity. The excellent results of the tested maize varieties in terms of lodging resistance, grain yield, grain color, number of cobs per single crop, and well-coverage of cob tips were noted by farmers as reasons for their appreciation and acceptance. Also, according to extension staff, this cluster-based outcome demonstration opened the door for farmers to implement full-package applications to boost the productivity of maize varieties. In addition, they stated that the linkage between research, farmers, and extension is the most effective method for addressing smallholder farmers' productivity problems and issues with food security. Extension workers claimed that the proven methods and methodology for growing maize have a remarkable effect on smallholder farmers' ability to produce more maize. To maintain the results and further raise agricultural output, careful consideration must be given to this link between research, farmers, and extension. Pole worm prevalence, however, poses a significant threat to maize production, so the study center must pay careful attention to this issue.

Challenges faced

Due to their nature, agricultural activities are difficult to carry out because they are done in an open or uncontrolled environment where they are highly susceptible to unanticipated circumstances and the real conditions of farmers and other stakeholders. (technical support, agronomic practices, and conditional attitudes related to personal benefit). The effects of the aforementioned factors collectively hamper agricultural productivity and production in general, in addition to deviating from an agricultural project's intended objective.

When carrying out these demonstration tasks, there were challenges, including an outbreak of pole worms, but they were overcome without having a negative impact as a result of the use of integrative pest management techniques and collaboration with farmers, extension agents, and researchers.

Additionally, taking yield samples and determining the appropriate grain yield weight was difficult due to the heavy rain that was falling during the maturity stage. Also, extension gatekeepers' requests for periodic incentives to monitor and organize demonstration field management are growing in number as a source of grievance. However, this difficulty can be overcome by opening up channels of communication to zone and woreda extension staff so they can plan out their demonstration supervision schedule

Lessons learned

The application of this cluster-based full demonstration approach validated the effective utilization of production factors (land, labor, and technologies) to boost smallholder farmers' output and productivity. By putting farmers at the center of the research-extension relationship and fostering effective communication, smallholder farmers can readily disseminate research findings and boost agricultural productivity.

Farmers thought that utilizing recommended full packages could boost the productivity of particular agricultural technology. Farmers were also extremely motivated and believed that using the recommended full packages could boost the productivity of maize technology and, to the greatest extent possible, demonstrate the variety's potential.

CONCLUSION

The demonstrated maize varieties (BH547 and BH661) were the best performed; their average grain yields were 44 quintals ha-1 and 42 quintals ha-1, respectively. The result of the demonstration showed that using the recommended full packages for the maize technologies (BH547 and BH661) could increase the production and

productivity of the varieties. Hence extending these improved maize varieties with their full packages is an important mechanism to increase the production volume and productivity of smallholder farmers up to 43 quintals per hectare in the demonstration and similar agro-ecologies. The grain yield, the number of cobs per plant, disease resistance capacity, seed color, and the well-covered seed of the varieties were preferred by the producers. Farmers were also extremely motivated and believed that using the recommended full packages could boost the productivity of maize technology and, to the greatest extent possible, demonstrate the variety's potential.

Recommendation

The demonstrated maize technologies resulted in positive change in farmers' maize productivities and showed profitable maize-producing practices, thus expanding this technology with its full package would play a great role in household-level food security and income generation for smallholder farmers and contribute to zonal and regional food security. Therefore, each concerned body needs to play its expected roles, in that manner: -

Farmers need to expand the technology as demonstrated in the packages and further refine agronomic practices (farming frequency, weed control) to maintain the productivity achieved in the demonstration as well as further improve grain yields by incorporating their indigenous knowledge, especially for the biological control of pole worm prevalence.

Also, cooperatives need to play their role in seed multiplication for the sake of seed access for farmers and other interested parties who are interested in working on this technology.

Agricultural officers need to play their role in facilitating communication among farmers, cooperatives, and researchers and giving technical support on agronomic practices (farming frequency, weed management, pest management, and rust prevention and control mechanisms). The pole worm prevalence in maize cultivars is a big production problem at demonstration locations; thus, concerned bodies need to give due attention to the solution to this problem.

Finally, all concerned parties (especially extension personnel) must pay particular attention to technical support and information accessibility for every smallholder maize producer for proper actualization of maize production packages in the area in order to maintain the demonstration's positive results and reduce the yield performance gap among farmers.

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Research Article

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Effect of Cassava-Legume Intercropping Systems on the Physicochemical Properties of the Soil in Three Agro-Ecological Zones of Sierra Leone

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ABSTRACT

The study aims at evaluating the effects of cassava-legume intercropping systems on major soil nutrients across three agro-ecological zones of the country. The experiment was arranged in a randomized complete block design (RCBD) with three replications. The treatments consisted of four cropping systems (sole cassava, cassava + cowpea, cassava + groundnut, and cassava + soybean).The study shows a general decrease in soil pH by 1.48-9.91% and 4.24-11.375% among the agro-ecological zones and cropping systems respectively. Organic carbon increased by 28.8% in the savannah woodland zone in Makeni but decreased by 9.69% and 40.37% in the rainforest zone in Segbwema and the transitional rainforest zone in Sumbuya respectively. It also decreased by 26.27%, 12.08%, and 0.92% for the sole cassava, cassava-cowpea, and cassava-groundnut systems respectively. It was however observed to increase by 10.97% for the cassava-soybean system in the rainforest zone in Segbwema. The total nitrogen, on the other hand, increased slightly by 1.11-1.73% across the agro-ecological zones and 2.62-10.84% for the cropping systems. Total nitrogen for the sole cassava was however observed to decrease by 14.31%. Available phosphorus decreased by 47.35-59.02% and 36.23-72.89% for the agro-ecological zones and the cropping systems respectively. In addition, exchangeable potassium also decreased by 33.33-38.42% and 25.26-49.985 % for the agro-ecological zones and the cropping systems respectively. In addition, the result shows strong, positive, and significant correlations between pH with organic carbon, pH with total nitrogen, organic carbon, and total nitrogen in the three agro-ecological zones.

INTRODUCTION

The sole production of cassava will impoverish the soil rapidly unless the absorbed or lost nutrients are replenished (Eke-Okoro et al., 1999). For example, an average of 660 kg/ ha, 75 kg/ ha, and 450 kg/ ha of Nitrogen, Phosphorus, and Potassium respectively, have been lost from approximately 200 million hectares of cultivated land in thirty-seven African countries (Smaling et al., 1997) in the last thirty years. As a result of this, the necessity to improve soil fertility through the inclusion of legumes into the cropping systems has therefore become a very important issue in the development policy agenda of most African governments due to the strong linkage between soil fertility and food security on the one hand, and the implications on the livelihood of the population on the other (Mugwe et al., 2011). As a result, crop scientists have recommended the inclusion of leguminous crops into crop production systems as a way to address the problem of declining soil fertility. Several studies have reported the advantages of cassava-legume mixtures, especially in improving the nitrogen content of

the soil through the fixation of atmosp heric nitrogen (Aigh, 2007). Kurtz (2006) reported significantly higher values of yield and yield components of cassava intercropped with grain legumes than those of the yield components of sole-cropped cassava. However, although legumes are known to fix nitrogen in the soil, studies have shown that the amount of nitrogen fixed depends on the species of the legume. For example, Peoples et al. (2009) reported that (73 – 354), (168 –208), (72 – 124), (55 – 168), and (40 – 65) kg N ha-1 was fixed into the soil by cowpea, pigeon pea, groundnut, and soybean respectively.

Of the sixteen essential plant nutrients needed for plant growth, development, and reproduction, nitrogen is the most important and the most easily limited or deficient throughout the world, particularly in the tropics (Agbede, 2009). The reason for the inadequate supply of nitrogen is the fact that nitrogen exists in organic form in the soil, which must be mineralized before it is used by plants (Azam, 2002). As such, legumes can convert free atmospheric nitrogen (N2) into ammonia (NH3) through the process called biological nitrogen fixation (BNF) with the help of specific bacteria (Rhizobium) which reside in the nodules of legumes. The plants will now thereafter transform it into a usable form of plant nitrogen such as amino acids and proteins.

Despite the potential for cassava-legume intercropping technology in addressing the soil nutrient depletion problems of smallholder farmers in some parts of Africa, this knowledge is lacking among the smallholder farmers in Sierra Leone. To this end, this study was mainly aimed at evaluating the effect of intercropping grain legumes with cassava on the major soil nutrients. It is hypothesized that there is a net decrease in the concentration of soil nutrients after the cultivation of sole cassava than when intercropped with legumes such as cowpea, soybean, and groundnut.

MATERIALS AND METHODS

Study area and soil

The study was conducted between 2015-2017 cropping seasons under rain-fed conditions in three agro-ecological zones namely Sumbuya (N 08.040880 , W 011.4789550) in Bo district representing the transitional rainforest, Makeni (N 08.87200 , W 012.03760) in Bombali district representing the savannah woodland and Segbwema (N 07.99300, W 010.952240) in Kailahun district representing the rainforest region of the country (Figure 1).

Land preparation

The land at the three zones was slashed with a cutlass, burnt, destumped, and dug using a hoe and the plots were laid out using a measuring tape, a garden line, and, pegs.

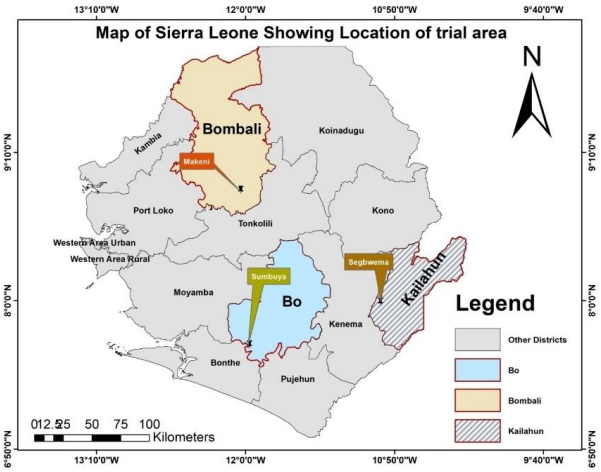

Figure 1 Map of Sierra Leone showing trial sites of the different agro-ecological zones in Sierra Leone

Experimental design, treatments and planting

The experiment was a factorial randomized complete block design with three replications. The treatments consisted of four cropping associations (sole cassava, cassava + cowpea, cassava + groundnut, and cassava + soybean) (Mansaray et al. 2022a). The plot size was 7m x 6 m.

The cassava and the three legumes were planted on a flat land in June of each year. Stem cuttings of about 25 cm long with five nodes were used. Cassava was planted at the spacing of 1 m x 1 m respectively; whilst cowpea and groundnut were planted at the spacing of 50 cm x 20 cm with two seeds per hole for cowpea and one seed per hole for groundnut. On the other hand, soybean was planted at the spacing of 50 cm x 10 cm with two seeds per hole. The legumes were introduced in between the rows of the cassava (Mansaray et al., 2022b).

The cassava variety used was slicass 6 whilst, the cowpea, soybean, and groundnut varieties used were IITA 573k-1-1, Slibean 2, and Slinut 1 respectively. Weeding was done with a hoe at one, three, and six months after planting (Mansaray et al., 2021). Cassava was harvested at 12 months after planting whilst the three legumes were harvested at their respective maturity dates. The haulms

of the harvested legumes were returned to the cassava system (Mansaray et al. 2021).

Soil sampling and laboratory analysis

Prior to planting, initial soil samples were collected at each of the three agro-ecological zones. In each zone, ten core soil samples from the topsoil (0- 20 cm) depth were collected in a "W" shaped design and mixed to form a composite sample. Additional soil samples were also collected per treatment at the harvesting stage of the cassava. The samples were air-dried, crushed, and sieve through a 2 mm sieve before analysis for chemical and physical properties using standard laboratory procedures. The soil properties determined were soil pH, total nitrogen, soil organic carbon, available phosphorus, exchangeable potassium, and soil particle sizes.

The soil pH in water (1:1 soil: water ratio) was determined using the pH meter; total nitrogen was determined using the procedure described by Bremner and Mulvaney (1982). The soil organic carbon was determined using the modified Walkley and Black's wet oxidation method as outlined by Nelson and Sommers (1982) whilst the available phosphorus was determined using the procedure described by Bray and Kurtz (1945). The exchangeable potassium was determined using the flame photometer. The soil particle sizes were determined using the hydrometer method described by Jones (2001).

Data analysis

Pearson correlation was performed among the soil chemical properties in order to establish relationship among them.

RESULTS

Initial soil properties

The soil in Makeni representing the savannah woodland was sandy clay loam in texture with a pH of 4.50. Organic carbon and total nitrogen were 67.60 t/ha and 8.06 t/ha respectively. Available phosphorus and exchangeable potassium were 6.60 mg/kg and 0.045 Cmol/kg respectively (Table 1). In Sumbuya representing the transitional rainforest, the soil was sandy loam in texture with a pH of 5.20; whilst organic and total nitrogen content was 114.40 t/ha and 10.67 t/ha respectively. The available phosphorus and exchangeable potassium were 24.50 mg/kg and 0.047 Cmol/kg respectively (Table 1). For Segbwema representing the rainforest, the soil was also sandy loam with a pH of 5.30. The organic carbon and total nitrogen content were 132.60 t/ha and 11.70 t/ha respectively. The available phosphorus and exchangeable potassium were 36.30 mg/kg and 0.054 Cmol/kg respectively (Table 1).

Table 1 The physicochemical composition of the soil in the three agro-ecological zones before establishing the trial.

Treatment	Agro-ecological zones		
	Savannah woodland (Makeni)	Transitional rainforest (Sumbuya)	Rainforest (Segbwema)
pH (Water)	4.50	5.20	5.30
Organic carbon (t/ha)	67.60	114.40	132.60
Total nitrogen (t/ha)	8.06	10.67	11.70
Available phosphorus (mg/kg)	6.60	24.5	36.30
Exchangeable potassium (Cmol/kg)	0.045	0.047	0.054
Electrical conductivity	115.00	266.00	66.00.00
Soil texture	Sandy clay loam	Sandy loam	Sandy loam
Sand (%)	71.40	77.36	65.40
Silt (%)	10.00	4.00	12.0
Clay (%)	18.60	18.64	22.6

Changes in soil nutrient status at harvest of the cassava at the three agro-ecological zones

Soil pH

The initial pH values for the agro-ecological zones in Makeni, Sumbuya, and Segbwema were 4.50, 5.20, and 5.30 respectively (Table 2). The pH at harvest of the cassava ranged from 4.30-6.60, 4.35-4.70, and 4.60-4.90 under all the cropping systems for the agro-ecological zones in Makeni, Sumbuya, and Segbwema respectively. At harvest of the cassava, the pH values were decreased under all the cropping systems in the three agro-ecological zones except for the cassava-soybean cropping system in the savannah woodland in Makeni, which recorded a slight increase in pH of 4.44% (Table 2). The highest percentage decrease in pH was recorded in the transitional rainforest zone in Sumbuya (12.77%) followed by the rainforest zone in Segbwema and the savannah woodland zone in Makeni (9.91%).

Concerning the cropping systems, the highest percentage decrease in pH was recorded for the sole cassava (11.37%) followed by cassava-cowpea (9.00%), cassava-

groundnut (7.09%), and cassava-soybean system (4.24%). In general, the soil pH under all the cropping systems and agro-ecological zones was strongly acidic.

Table 2 Effect of cropping systems on soil pH in the three agro-ecological zones over two cropping seasons

Soil pH (1:1 H_20)													
	Agro-ecological zone												
	Savannah woodland (Makeni)				Transitional rainforest (Sumbuya)				Rainforest (Segbwema)				Mean (%)
	Initial	Final	Change	% Change	Initial	Final	Change	% Change	Initial	Final	Change	% Change	
Cropping system													
Sole Cassava	4.50	4.30	-0.20	-4.44	5.20	4.35	-0.85	-16.46	5.30	4.60	-0.70	-13.21	**-11.37**
Cassava-cowpea	4.50	4.40	-0.10	-2.22	5.20	4.50	-0.70	-13.46	5.30	4.70	-0.60	-11.32	**-9.00**
Cassava-groundnut	4.50	4.40	-0.10	-2.22	5.20	4.60	-0.60	-11.53	5.30	4.90	-0.40	-7.54	**-7.09**
Cassava-soybean	4.50	4.60	0.20	4.44	5.20	4.70	-0.50	-9.61	5.30	4.90	-0.40	-7.54	**-4.24**
Mean				**-1.48**				**-12.77**				**-9.91**	

Soil organic Carbon

Soil organic carbon increased at the harvest of the cassava across all the cropping systems in the savannah woodland zone in Makeni by 28.84% (Table 3). However, it was observed to decrease harvest of the cassava by 40.37% and 9.69% in the transitional rainforest zone in Sumbuya and the rainforest zone in Segbwema respectively. For the cropping systems, the percentage decrease in soil organic carbon at harvest of cassava was higher for the sole cassava (26.27%) followed by the cassava-cowpea (12.08%), and the cassava-groundnut system (0.92%) (Table 3). On the other hand, soil organic carbon was on average higher by 10.92% at harvest of the cassava for the cassava-soybean cropping system.

Table 3. Effect of cropping system on soil organic carbon in three agro-ecological zones over two cropping seasons

Soil organic carbon (t/ha)													
	Agro-ecological zone												
	Savannah woodland (Makeni)				Transitional rainforest (Sumbuya)				Rainforest (Segbwema)				Mean (%)
	Initial	Final	*Change	% Change	Initial	Final	*Change	% Change	Initial	Final	*Change	% Change	
Cropping system													
Sole Cassava	67.60	70.20	2.60	3.84	114.40	59.80	-54.60	-47.77	132.60	85.80	-46.20	-34.84	**-26.27**
Cassava-cowpea	67.60	88.40	20.80	30.76	114.40	62.40	-52.00	-45.45	132.60	104.00	-28.60	-21.56	**-12.08**
Cassava-groundnut	67.60	91.00	23.40	34.61	114.40	65.00	-49.40	-43.26	132.60	140.40	7.80	5.88	**-0.92**
Cassava-soybean	67.60	98.80	31.20	46.15	114.40	85.80	-28.60	-25.00	132.60	148.20	15.60	11.76	**10.97**
Mean				**28.84**				**-40.37**				**-9.69**	

Total soil Nitrogen

The total soil nitrogen content at harvest of the cassava was observed to increase in the three agro-ecological zones and the cropping systems except for the sole cassava system (Table 4). The percentage increase in the total soil nitrogen content was higher in the rainforest zone in Segbwema (1.73%) followed by the transitional rainforest zone in Sumbuya (1.24%) and the savannah woodland zone in Makeni (1.11%) (Table 4). In the case of the cropping system, the highest increase in the total nitrogen was recorded for the cassava-soybean system (10.84%) followed by the cassava-groundnut system (6.27%), cassava-cowpea system (2.62%), and the sole cassava (14.31%) across the three agro-ecological zones (Table 4).

Table 4. Effect of cropping system on the total soil nitrogen in three agro-ecological zones over two cropping seasons.

Soil total nitrogen (t/ha)													
	Agro-ecological zone												
	Savannah woodland (Makeni)				Transitional rainforest (Sumbuya)				Rainforest (Segbwema)		Mean (%)		
	Initial	Final	*Change	% Change	Initial	Final	*Change	% Change	Initial	Final	*Change	% Change	
Cropping system													
Sole Cassava	8.06	6.10	-1.96	-24.32	10.66	9.36	-1.30	-12.19	11.70	10.95	-0.75	-6.41	**-14.31**
Cassava-cowpea	8.06	8.32	0.26	3.22	10.66	10.92	0.26	2.43	11.70	11.96	0.26	2.22	**2.62**
Cassava-groundnut	8.06	8.84	0.76	9.42	10.66	11.18	0.53	4.97	11.70	12.22	0.52	4.44	**6.27**
Cassava-soybean	8.06	9.36	1.30	16.12	10.66	11.70	1.04	9.75	11.70	12.48	0.78	6.66	**10.84**
Mean				**1.11**				**1.24**				**1.73**	

Soil available Phosphorus

There was a general reduction in soil available phosphorus concerning the cropping systems in the three agro-ecological zones (Table 5). The percentage reduction across the cropping systems ranged from 16.67-71.71%, 44.90-70.61%, and 47.77-76.86% for the agro-ecological zones in Makeni, Sumbuya, and Segbwema respectively. For the agro-ecological zones, Sumbuya in the transitional rainforest (58.67%) recorded the highest reduction followed by Segbwema in the rain forest (52.75%), and Makeni in the savannah woodland (47.37%). Pertaining to the cropping systems, the highest reduction in available phosphorus was recorded for the cassava-soybean system (72.89%) followed by the cassava-groundnut system (56.54%), cassava-cowpea (54.39%), and the sole cassava (36.23%) (Table 5).

Table 5 Effect of cropping system on the soil available phosphorus in three agro-ecological zonesover two cropping seasons

Available phosphorus (mg/kg)													
	Agro-ecological zone												
	Savannah woodland (Makeni)				Transitional rainforest (Sumbuya)				Rainforest (Segbwema)				Mean (%)
	Initial	Final	*Change	% Change	Initial	Final	*Change	% Change	Initial	Final	*Change	% Change	
Cropping system													
Sole Cassava	6.60	5.50	-1.10	-16.67	24.50	13.50	-11.00	-44.90	36.30	19.20	-17.10	-47.11	**-36.23**
Cassava-cowpea	6.60	3.90	-2.70	-40.90	24.50	7.30	-17.20	-70.20	36.30	17.40	-18.90	-52.07	**-54.39**
Cassava-groundnut	6.60	2.60	-4.00	-60.60	24.50	12.50	-12.00	-48.98	36.30	14.50	-21.80	-60.05	**-56.54**
Cassava-soybean	6.60	1.90	-4.70	-71.21	24.50	7.20	-17.30	-70.61	36.30	8.40	-27.90	-76.86	**-72.89**
Mean				**-47.35**				**-58.67**				**-59.02**	

Soil exchangeable Potassium

Similarly, there was a general decrease in exchangeable potassium across the cropping systems and agro-ecological zones (Table 6). The percentage reduction was higher in the rainforest zone in Segbwema (38.42%) followed by the transitional rainforest zone in Sumbuya (37.77%) and the savannah woodland zone in Makeni (33.33%). The percentage reduction in exchangeable potassium ranged from 22.22-48.88%, 27.66-51.06%, and 25.00-50.00% for the agro-ecological zones in Makeni, Sumbuya, and Segbwema respectively. In the case of the cropping systems, the highest mean percentage reduction was recorded for the cassava-soybean system (49.98%) followed by the cassava-groundnut system (39.64%), the cassava-cowpea system (31.13%), and the sole cassava (25.26%) (Table 6).

Table 6 Effect of cropping system on the soil exchangeable potassium in three agro-ecological zones over two cropping seasons

Exchangeable potassium (Cmol/kg)													
	Agro-ecological zone												
	Savannah woodland (Makeni)				Transitional rainforest (Sumbuya)				Rainforest (Segbwema)				Mean (%)
	Initial	Final	*Change	% Change	Initial	Final	*Change	% Change	Initial	Final	*Change	% Change	
Cropping system													
Sole Cassava	0.045	0.035	-0.010	-22.22	0.047	0.034	-0.013	-27.66	0.054	0.040	-0.014	-25.92	**-25.26**
Cassava-cowpea	0.045	0.034	-0.011	-24.44	0.047	0.032	-0.015	-31.91	0.054	0.034	-0.020	-37.03	**-31.13**
Cassava-groundnut	0.045	0.028	-0.017	-37.77	0.047	0.028	-0.019	-40.42	0.054	0.032	-0.022	-40.74	**-39.64**
Cassava-soybean	0.045	0.023	-0.022	-48.88	0.047	0.023	-0.024	-51.06	0.054	0.027	-0.027	-50.00	**-49.98**
Mean				**-33.33**				**-37.77**				**-38.42**	

Correlation among soil chemical properties across the agro-ecologicalzones

From the results, a strong, positive, and significant correlation was recorded between pH with soil organic carbon in the savannah woodland zone in Makeni (P = 0.0017, r = 0.8023), the transitional rainforest zone in Sumbuya (P = 0.0019, r = 0.8621), and the rainforest zone in Segbwema (P = 0.0002, r = 0.8821) (Table 7). For soil total nitrogen, a strong, positive, and significant correlation was also recorded between pH with soil total nitrogen for the agro-ecological zones in Makeni (P = 0.0014, r = 0.8246), Sumbuya (P = 0.0016, r = 0.8446), and Segbwema (P = 0.0002, r = 0.8712) (Table 7).

In the case of the correlation between soil organic carbon with total nitrogen, a very strong, positive, and significant correlation was recorded for both agro-ecological zones in Segbwema (P = 0.0069, r = 0.9930) and Sumbuya (P = 0.0443, r = 0.9257) whilst, a moderately strong, positive, and significant correlation was recorded in the savannah woodland zone in Makeni (P = 0.0045, r = 0.7245) (Table 7).

Table 7 Correlation matrix among soil chemical properties in three agro-ecological zones

Pearson Correlation Coefficients Prob> |r| under H0: Rho=0															
	Agro-ecological zone														
Soil chemical properties	Savannah woodland					Transitional rainforest					Rainforest				
	pH	SOC	TN	AP	EP	pH	SOC	TN	AP	EP	pH	SOC	TN	AP	EP
pH	1.00	0.80 0.001	0.82 0.001	-0.86 0.13	-0.72 0.2765	1.00	0.88 0.0002	0.87 0.0002	-0.32 0.67	0.23 0.76	1.00	0.86 0.002	0.84 0.002	0.21 0.78	-0.04 0.95
SOC	0.80 0.002	1.00	0.72 0.005	-0.96 0.03	-0.59 0.40	0.88 0.0002	1.00	0.99 0.007	-0.90 0.09	-0.89 0.101	0.86 0.002	1.00	0.92 0.04	-0.85 0.14	-0.93 0.27

TN	0.82 0.002	0.72 0.005	1.00	-0.96 0.03	-0.57 0.42	0.87 0.00002	0.99 0.007	1.00	-0.80 0.19	-0.98 0.01	0.84 0.002	0.92 0.04	1.00	-0.72 0.27	-0.85 0.14
AP	-0.86 0.13	-0.96 0.03	-0.96 0.03	1.00	0.76 0.23	-0.32 0.67	-0.90 0.09	-0.80 0.19	1.00	0.84 0.15	0.21 0.78	-0.85 0.14	-0.72 0.27	1.00	0.72 0.27
EP	-0.72 0.27	-0.59 0.40	-0.57 0.42	0.76 0.23	1.00	0.23 0.76	-0.89 0.10	-0.98 0.013	0.84 0.15	1.00	-0.04 0.95	-0.93 0.06	-0.85 0.14	0.72 0.27	1.00

DISCUSSION

Soil nutrients are essential for plant growth and development. The results from the study show that cassava-legume intercropping systems can affect the soil in terms of pH, organic carbon, total nitrogen, available phosphorus, and exchangeable potassium.

The soil pH at harvest in the three agro-ecological zones and cropping systems was very strongly acidic. There was a decrease in pH of the soil concerning cropping systems and agro-ecological zones, which could be attributed probably to the removal of large quantity of nutrients from the soil especially, bases by the component crops. This result concord with the findings of Minhas et al. (1995) who reported a reduction in the mean pH from 6.7-5.7 when cassava was intercropped with soybean. The reduction in pH was higher for the agro-ecological zones in Sumbuya and Segbwema compared to Makeni probably because higher yields of the component crops were recorded in both zones compared to Makeni. Furthermore, the sole cassava recorded a higher reduction in pH compared to the intercropping systems probably because this system extracted more soil nutrients in the form of bases from the soil than it added to it. On the contrary, the slight increase in pH recorded in the savannah woodland in Makeni for the cassava-soybean cropping system suggests that intercropping could improve soil pH. The reason for the observed increase according to Cong and Merckx (2005) could be probably due to the transformation of nitrogen and the release of metal ions resulting from the decomposition of organic residues. Furthermore, Matusso et al. (2012) and Owusu and Sadick (2016) argued that, the increase in soil pH value in intercropping systems shows that intercropping could decrease soil acidity as a result of higher organic matter production.

This observation concord with the findings of Esekhade and Idoko (2010) who reported higher soil pH in the legume-cereal intercropping system compared to their counterpart under the mono-cropping system. In addition, Schoenerberger et al. (2002) reported changes in soil pH from strongly acidic to slightly acidic in the maize-legume intercropping system.

The result further reveals a strong, positive, and significant correlation between pH with soil organic carbon, and between pH with total nitrogen; indicating that the higher the pH the greater the availability of these nutrients to the plant. This further shows that the availability of these two soil nutrients were strongly affected by soil pH; as it determines the variation of soil microorganism community structure and diversity (Tripathi et al., 2018), which controls the process of decomposition and mineralization of soil organic matter and the subsequent released of nutrients to plants. Furthermore, Rousk et al. (2010) showed that the relative abundance and diversity of bacteria were positively related to pH. This effect impacts the mineralization process leading to higher nitrogen content in soils with higher pH. This result concord with the findings of Xu et al. (2019) who reported positive correlations between soil organic carbon and pH in central-eastern Europe. On the contrary, Reisser et al. (2016) reported a general negative correlation between organic carbon and nitrogen with pH under natural conditions at various sites. This suggests that a relatively low pH favours the accumulation of organic matter (Zhou et al., 2019). This negative correlation shows that high pH values tend to have lower soil organic carbon content and total nitrogen whilst low pH tends to have higher soil organic carbon and total nitrogen content. The reason adduced by these authors for the negative correlation is that soil organic matter upon decomposition releases organic acids which lead to low pH value. Soil pH is a major driver controlling

nutrient availability for plants and thus, influences biomass production indirectly (Bolan et al., 2003; Wang et al., 2012).

Soil organic carbon is one of the key attributes in assessing soil health; it is generally positively correlated with crop yield (Bennett et al., 2010). Murphy (2015) opined that, important functional processes in soil such as the ability to store nutrients especially, nitrogen, water holding capacity, and aggregate stability are strongly influenced by the organic carbon content in the soil. It is also important for increased agricultural production because organic matter helps improve soil structure and cation exchange capacity and hold water; thus, it has a positive impact on soil fertility (West and Post, 2002).

From the result, soil organic matter was observed to increase across cropping systems in the savannah woodland in Makeni probably due to the decomposition of a lot of biomass returned from cassava and component crops during the growing season as reported by Ojeniyi and Adetoro (1993) who noted an appreciable increase in soil organic carbon following the decomposition of leaves of Gliricidia sepium. The authors adduced the increase in soil organic carbon after cropping to the high rate of mineralization informed by the fast rate of decomposition of legume leaves due to their low C: N ratio. Moreover, the increase in soil organic carbon at harvest could also be because the cultivation of cassava may have minimized erosion and microbial decomposition rate considerably while maintaining favorable soil moisture conditions. According to King et al. (2019), regardless of the decomposition rates, where organic inputs outweigh organic matter losses, soil organic carbon should increase even though slowly.

This result conforms to the findings of Matusso et al. (2012) who reported higher soil organic matter with intercropping. In addition, Ispandi (2002) reported an increase in organic carbon of 12% and 56% when cassava was intercropped with maize and groundnut respectively. Similarly, Nath et al. (2003), Aulakh et al. (2004), and Swain (2016) have also reported an increase in the organic carbon content of orchard soil due to intercropping practices in fruit orchards.

On the contrary, there was a depletion of soil organic carbon for the agro-ecological zones in Sumbuya and Segbwema probably because of higher nutrient uptake by component crops than the quantity of nutrients supplied through the legumes (Jones, 2016). Another possible reason could be due to higher yields of component crops reported for these agro-ecological zones. This result concord with the findings of Yan et al. (2006) who reported the possibility of rapid nutrient depletion under intercropping systems due to the combined demands of the individual intercrops for nutrients.

Furthermore, soil organic carbon was observed to decrease among the cropping systems except for the cassava-soybean system which recorded an increase in soil organic carbon. The rate of soil organic carbon depletion was higher for the sole cassava probably because cassava being a wide-spaced crop, most of the soil was left vacant under the sole cropping system resulting in a higher loss of soil organic matter by oxidation and less addition of soil biomass. The higher soil organic carbon recorded concerning the cassava-soybean system could be because a higher quantity of nutrients was supplied to the system through the legumes than the amount of nutrient that was taken up by the component crops. This result agrees with the findings of Akinnifesi et al. (2007) and Sebetha (2015) who reported an increase in soil organic carbon under the cereal-legume intercropping system.

The result further reveals a strong, positive, and significant correlation between soil organic carbon and total nitrogen across the three agro-ecological zones. This shows that an increase in soil organic carbon will be followed by an increase in total nitrogen as reported by Brevik et al. (2018). This result concord with the findings of Sadovnikova et al. (1996) who reported a strong, positive correlation between soil organic carbon with total nitrogen.

Pertaining to the total nitrogen, there was a general increase in the total nitrogen content across cropping systems in the three agro-ecological zones except for the sole cassava, which recorded a decrease in total nitrogen content at harvest of the cassava. The increase in total nitrogen content across the agro-ecological zones was generally slight, probably due to the excessive removal of nitrogen by cassava for root formation in all the zones. The general decrease in the total nitrogen for the sole cassava system could be because cassava removes a large amount of nitrogen from the soil for root yield formation as reported by Obigbesan (1977) and CIAT (1982). Furthermore, it has been reported by Padwick (1983) that African soils show nutrient-deficiency problems after a short period of cultivation, with nitrogen being the most

rapidly depleted nutrient. Other possible reasons for the observed depletion of nitrogen under the sole cassava system could be because cassava is normally planted in a wide spacing at the start of the rainy season when the soil is exposed and has not been covered by a canopy and thus, susceptible to erosion.

On the other hand, the observed increase in total nitrogen content across the intercropping systems could be because legumes have the ability to trap and fix nitrogen into the soil through their root nodules as reported by Crews and Peoples (2004). Furthermore, the large amount of biomass produced by both the cassava and the legumes after mineralization could have released a large amount of nitrogen into the soil. This result corroborates the findings of Nweke (2016) who reported significant levels of nitrogen in plots containing maize that was intercropped with groundnut. Similar results of an increase in the available nitrogen content of the soil through intercropping in mango orchards have been reported by Swain (2016).

Furthermore, Nnadi and Haque (2017) have shown that legumes might contribute about 30% N from the nitrogen fixation process to other crops in intercropping and crop rotation. Bundy and Andraski (2005) also reported that the residues of corn returned to the field can contribute 50-100 kg N/ha where 5-20% of nitrogen residue can still be used by the next crop (Bundy and Andraski, 2005).

Concerning available phosphorus, there was a general reduction across cropping systems in the three agro ecological zones. The reason for the general decline in available phosphorus could be related probably to the fact that the component crops may have taken up a large amount of phosphorus from the soil. The above observation agrees with the findings by Onwueme and Sinha (1991). These authors reported that root crops take up more Phosphorus and Potassium from the soil than any other crop species. The reduction in available phosphorus was higher for the agro-ecological zones in Segbwema and Sumbuya probably due to the higher yields of component crops reported in the two agro-ecological zones. Furthermore, the reported decrease in available phosphorus of the intercrops compared to the sole cassava could be attributed to the excessive demand and use of phosphorus by legumes for nitrogen fixation and other physiological processes. The result conforms to the findings of Nweke and Emeh (2013) who reported that legumes required an abundant amount of phosphorus in the soil for nitrogen fixation and growth. This result is in contrast to the findings of Carel (2006) who reported an increase in soil-available phosphorus under intercropping involving legumes and adduced this to the mineralization of organic phosphorus, which in turn, results in the release of more phosphorus for crop use.

Similarly, a general reduction in exchangeable potassium was recorded across cropping systems in the three agro-ecological zones probably because there is always a high demand for nutrients by component crops in intercropping systems (Yahaya et al., 2014). Similar observation was also reported by Kurt (1984). The depletion of exchangeable potassium was more severe in the rainforest zones in Segbwema and the transitional rainforest zone in Sumbuya compared to the savannah woodland zone in Makeni probably due to the higher tuberous root yield that was produced at these two zones as potassium is the most limiting factor in cassava nutrition. The indispensability of potassium in cassava nutrition had been demonstrated by many studies (Nyi, 2014; Pypers et al., 2011). This result agrees with the findings of Bharabwaj et al. (1994) who reported that the uptake of potassium by crops generally increases with an increase in crop yield.

Furthermore, the higher rate of potassium depletion recorded with respect to the intercropping system compared to the sole cassava could be probably due to the removal of potassium from the soil by both the cassava and component crops. Another factor that can be implicated in the decrease in exchangeable potassium is the inability of the legumes to fix appreciable quantities of potassium into the soil, unlike nitrogen, as legumes are generally known for nitrogen fixation. This result concord with the finding of Yahaya et al. (2014) who reported rapid nutrient depletion under intercropping systems due to the combined demands of the individual intercrops for nutrients. The decrease among intercropping systems was higher for the cassava-soybean system probably because of the higher root yield recorded for this cropping system in the three agro-climatic zones.

CONCLUSIONS

The result of this study has established that intercropping cowpea, soybean, and groundnut with cassava decreased soil pH among cropping systems in the three agro-ecological zones except for the cassava-soybean system

in the savannah woodland zone in Makeni. Soil organic carbon increased in the savannah woodland zone in Makeni but decreased in the rainforest zone in Segbwema and the transitional rainforest zone in Sumbuya among the cropping systems except for the cassava-soybean system. Soil total nitrogen increased across cropping systems in the three zones except for the sole cassava. Exchangeable potassium and available phosphorus decreased under all cropping systems at all three zones. Furthermore, a strong, positive correlation was observed between organic carbon and total nitrogen with pH on the one hand, and between organic carbon and total nitrogen on the other.

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Authors' Contributions

All authors contributed equally to the conception and design of the study.

Competing Interests

The authors have declared that no competing interests exist.

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Assessment of Microbial Isolates in Date Palm Plantation Soils of Modibbo Adama University, Yola Adamawa State, Nigeria

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ABSTRACT

This research assessed the microbial isolates in the soil of the date palm plantation of Modibbo Adama University, Yola, Adamawa State. Parameters evaluated included; microbial isolates based on the growth performances of the date palm. The plantation was divided according to growth variabilities. Fifteen (15) auger points were taken, five (5) in each performance site based on the corresponding variability as observed. Soil samples were collected within the same points for analysis of microbial isolates, Nutrient agar medium at 10^5 dilutions was inoculated in a petri-dishes and incubated at 300C±10°C for 2-5 days for bacteria colonies, while for fungi and actinomycetes, sabaurond dextrose agar was used at 25°C for 5-7 days and afterward microorganisms per colony forming units (Cfu) were counted. Results from the study area showed that the area is highly rich in microorganisms. A total of 5096 colonies were scattered within 10 families in the study area. Some microorganisms identified were Actinomyces crime, Aspergillus niger, Staphylococcus aurus, Streptococcus species, Pseudomonas auroginosa, Escherichia coli, Bacillus subtilis, and Lactobacillus species among others. The finding of this study revealed that there was no significant difference among the microorganism across the study site leading to the conclusion that soil microorganisms were present in the study area. However, the microorganisms were very active in the date palm plantation soil. This is an indication that a strong relationship exists between, microorganisms and the date palm trees.

INTRODUCTION

Soil contains many micro flora and fauna as long as there is a carbon source for energy. A large number of bacteria in the soil exist, but because of their small size, they have a smaller biomass. Actinomyces are 10 times smaller in number but are larger in size so they are similar in biomass to bacteria in soil (Bhattarai, et al. 2015; Roohallah et al. 2022).

Soil microbes, bacteria, archaea, and fungi play diverse and often critical roles in these ecosystem services. The vast metabolic diversity of soil microbes means their activities drive or contribute to the cycling of all major elements (e.g. C, N, P), and this cycling affects the structure and the functions of soil ecosystems as well as the ability of soils to provide services to people

provisioning and regulating ecosystem services (Suzanne ,2017). Plant and animal detritus and root exudates represent essential sources of energy and nutrients for soil microbial and faunal communities. Bacteria and fungi represent 95% of the biomass present in most soils, where they interact with a combination of micro-fauna (nematodes, protozoa), Meso-fauna (acari, Collembola, mites) and macro-fauna (earthworms, termites, molluscs) in complex soil food-web systems that determine the turnover of organic matter and associated nutrients in the soil environment (Moghimian and Kooch, 2013; Duran et al. 2019).

Decomposition of organic carbon in soil is driven primarily by the activities of bacteria and fungi, while only 10–15% of soil carbon flux can be directly attributed to the actions of fauna (Shang et al. 2017). The vast majority of soil microorganisms are heterotrophs that rely on organic matter for energy and nutrients. These can be divided into microorganisms that respond primarily to the addition of fresh carbon substrates (zymogenous or r-selected biomass) and those that derive their energy mainly from the decomposition of older, more recalcitrant forms of organic carbon (autochthonous or K-selected biomass) (Shang et al. 2017).

The date palm (*Phoenix dactylifera* L.) tree belongs to the family Arecaceae and is considered a symbol of life in the desert, as it tolerates high temperatures, water stress, and salinity more than many other fruit crops (Effi, et al. 2011). Date palms can be planted in a wide range of soils with varying amounts of organic and mineral nutrients. Many parts of the world where date palm is grown still follow the traditional mixed planting of dates of various ages at irregular spacing. Moreover, inadequate fertilizer application and lack of proper tree and bunch management, such as pruning and fruit thinning, lead to the production of low fruit quality and thus lower market values (Elamin et al. 2017).

In the five years of the establishment of the date palm plantation of Modibbo Adama University of Yola, studies have not been done on biological constituents of the soil. The growth of the individual date palm plants have not been uniform, while some are performing very well, others have indication of stunted growth. Since the growth of every plant depends largely on the nutrient status which is in turn affected by the activities of soil microorganism, information on the soil biological components in the date palm plantation becomes a pre requisite to understanding the differences in the performances of individual plants. The aim of this study is to assess the microbial isolates in the study area. The specific objectives are to identify and evaluate the microbial isolates in the study area.

At the end of the study the biological component of the date palm plantation has been ascertained. The information on microorganism will thus, baseline information for future management of the date palm plantation soils and by extension any other date palm that may be grown under similar conditions. The results of the research will be an invaluable tool to the date palm plantation managers in the Department of Forestry and Wildlife Management, Modibbo Adama University of Yola and indeed many other organizations and individuals that are involved in date palm research and production.

MATERIALS AND METHODS

The Study Area

Adamawa State is located in the North Eastern part of Nigeria. It lies between latitude 7o and 11o N of the equator and longitude 11o and 14o E (Figure 1) (Adebayo et al., 2020). The date palm plantation of the Department of Forestry and Wildlife Management Modibbo Adama University, Yola, Adamawa State is located between latitude 8°N and 11°N Longitude 11.5°E and 13.5°E (Figure 2). Adamawa state falls under the Sudan, southern and Guinea savannah types of vegetation and its experiences distinct dry and wet seasons with temperature and humidity varying with seasons. The wet or rainy season falls between April and November, which is characterized by a single maximum in August and September. During this season, the moisture-laden southwest trade wind from the Atlantic Ocean blows over the area. Seventy percent of the total rainfall in the area happen to fall within four month of May- September (Adebayo et al. 2020).

The area has an average of 62 rainy days, while average amount of rainfall recorded in the area is 972 mm the dry season which is the harmattan period between December March. The period is characterized by dry, dusty and hazy northern trade wind that blows over the area from Sahara desert. Temperature within the area varies with season. Although the temperatures are relatively high almost all the year round, temperature of the area ranges from 27°C-40° C. December and January is the coldest months with the average temperature of 34° C (Adebayo, et al. 2020). The natural vegetation of the area is Sudan savannah type which is characterized by thick vegetation around hills and mountain ranges. The vegetation has a wide variety of savannah trees species among which area are; Acacia spp, Adansonia spp, Anogeisus spp. (Akosim et al. 2020).

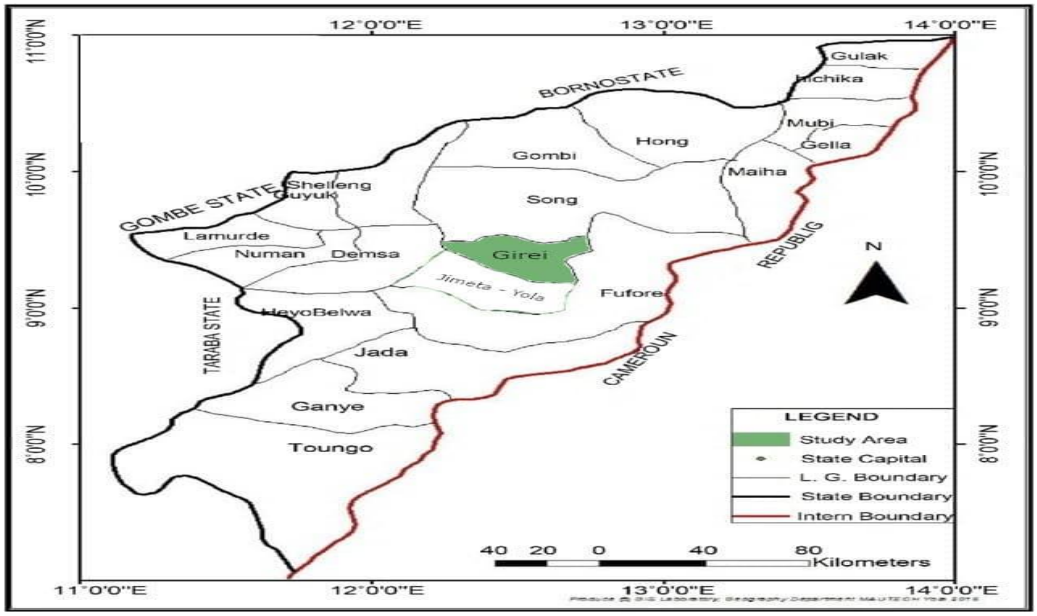

Figure 1: Map of Adamawa State showing Girei Local Government Area
Source: GIS Laboratory, Geography Department MAU, Yola (2022)

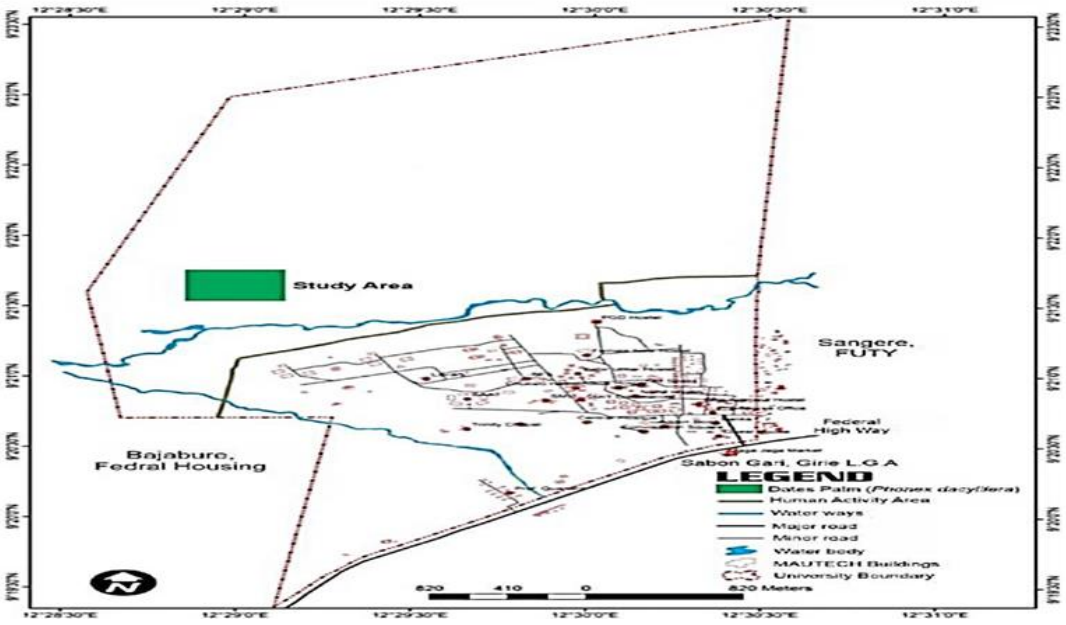

Figure 2: Map of MAU, Yola showing the study area
Source: GIS Laboratory, Geography Department MAU, Yola (2022).

The soils of Adamawa State are classified as ferruginous tropical soils. These types of soils are defined often generally as having a marked differentiation of horizons and an abundance of free iron oxides usually deposited as red or yellow mottles or concretions. The soils of Adamawa State as derived from this system include Luvisols, Legosols, Cambisols, Vertisols and Lithosols (Adebayo et al. 2020).

Soil sampling process for microbial isolates

Soil sample was taken in each auger point in the date palm plantation. A composite sample, after mixing the sample thoroughly, sterile polythene bags was used to convey samples to the laboratory within 24 hours of collection for analysis of soil bacteria, fungi and Actinomycetes at the department of microbiology of Modibbo Adama University, Yola.

Culture

Bacteria population was estimated by the method of Vieira, (2005) using the nutrient agar medium at 105 dilutions. The inoculated petri-dishes was incubated at 300c±10°c for 2-5 days for bacteria colonies. The laboratory analysis involved adding 1g of soil into 9ml of sterile water in a test tube, followed by vigorous shaking, and then serial dilution was done in four test tubes before

transfer into the petri dish. However, molten agar/media was poured into the petri dish.

For isolation and characterizing of fungi and Actinomycetes dilution plate method was used, sabaurond dextrose agar for fungi and Actinomycetes selected media for Actinomycetes was used as basal medium to isolate species. The inoculated petri- dish was incubated at 25°c for 5-7 days for growing the fungi and Actinomycetes colonies. Representative isolates of fungi was identified under the microscope with the help of standard manuals (Naher et al. 2013).Representative isolates of bacteria was also identified under the microscope. Fungi identification was done under appearance and pigmentation of spores on agar and Actinomycetes was identified under appearance on agar plates.

Data Analysis

Frequency tables were used in presenting the list of micro and macro species.

$$\text{Frequency} = \frac{\text{Number of individual specie occurrence}}{\text{Total number of all species}} \times 100$$

RESULTS

Soil Micro - organisms (cfu-1) in the Study Areas

The soil micro - organisms 'colony count result shows the presence of Actinomycetaceae, (*Actinomyces cream*, *Actinomyces yellow*, *Actinomyces blue*), Staphylococcaceae; (*Staphylococcus aurus*), Streptococcaceae, (*Streptococcus* spp.), Pseudomonadaceae, (*Pseudomonas auroginosa*), Enterobacteriaceae, (*Escherichia coli*), Bacillaceae, (*Bacillus subtilis*), Trichocomacaceae, (*Aspergillus niger*, *Aspergillus fumigates*), Lactobacillaceae, (*Lactobacillus* spp.), Aeromonadaceae, (*Aeromonas* spp.), Streptomycetaceae, (*Strestomycetes* spp.), Enterobacteriaceae, (*Klebsiilla* spp., *Proteus* spp., *Citrobacter* spp.), and Bacillaceae, (*Bacillus copus*). The soil micro - organism's colony where categorized into three (3) basic forms Bacteria, Fungi and Actinomycetes.

Table 1: Soil Micro - organisms (cfu-1) based on growth performance in the Study Area

Location	Bacteria (cfu^{-1})	Fungi (cfu^{-1})	Actinomycetes (cfu^{-1})
High			
P1	4.5×10^{-4}cfu/ml	3.5×10^{-4}cfu/ml	19.0×10^{-4}cfu/ml
P2	3.0×10^{-4}cfu/ml	4.8×10^{-4}cfu/ml	12.0×10^{-4}cfu/ml
P3	4.0×10^{-4}cfu/ml	3.5×10^{-4}cfu/ml.	16.0×10^{-4}cfu/ml
P4	4×10^{-4}cfu/ml.	5.4×10^{-4}cfu/ml	18.0×10^{-4}cfu/ml
P5	5.0×10^{-4}cfu/ml	3.0×10^{-4}cfu/ml	9.0×10^{-4}cfu/ml
Medium			
P1	3.8×10^{-4}cfu/ml	5.1×10^{-4}cfu/ml	6.0×10^{-4}cfu/ml.
P2	6.8×10^{-4}cfu/ml	4.7×10^{-4}cfu/ml	20.0×10^{-4}cfu/ml
P3	5.0×10^{-4}cfu/ml	4.6×10^{-4}cfu/ml	7.0×10^{-4}cfu/ml
P4	6.8×10^{-4}cfu/ml	4.9×10^{-4}cfu/ml.	9.0×10^{-4}cfu/ml
P5	5.0×10^{-4}cfu/ml	6.0×10^{-4}cfu/ml.	21.0×10^{-4}cfu/ml
Low			
P1	3.7×10^{-4}cfu/ml	5.5×10^{-4}cfu/ml.	16.0×10^{-4}cfu/ml
P2	4.5×10^{-4}cfu/ml	5.0×10^{-4}cfu/ml	11.0×10^{-4}cfu/ml
P3	4.5×10^{-4}cfu/ml	6.7×10^{-4}cfu/ml	16.0×10^{-4}cfu/ml
P4	3×10^{-4}cfu/ml.0	5.4×10^{-4}cfu/ml	18.0×10^{-4}cfu/ml
P5	3.5×10^{-4}cfu/ml	3.0×10^{-4}cfu/ml	9.0×10^{-4}cfu/ml

Source: Field Survey, (2022).
Key: Cfu = microorganisms per colony forming units

The result of the soil micro - organism's colony count (cfu-1) based on high growth performance in the study area is presented in Table 1. The results of the high growth performance site indicate that Bacteria count ranged from 3.0×10^{-4}cfu/ml to 5.0×10^{-4}cfu/ml, Fungi count ranged from 3.0×10^{-4}cfu/ml to 5.4×10^{-4}cfu/ml and Actinomycetes count also ranged from 9.0 ×10-4cfu/ml to 19.0×10^{-4}cfu/ml respectively.

Results of the medium growth performance site indicate that Bacteria count ranged from 3.8×10^{-4}cfu/ml to 6.8×10^{-4}cfu/ml, Fungi count ranged from 4.6×10^{-4}cfu/ml to 6.0×10^{-4}cfu/ml and Actinomycetes count also ranged from 6.0×10^{-4}cfu/ml to 21.0×10^{-4}cfu/ml respectively.

From the low-performance site, results indicate that Bacteria count ranged from 3.0×10^{-4}cfu/ml to 4.5×10^{-4}cfu/ml, Fungi count ranged from 3.0×10^{-4}cfu/ml to 6.7×10^{-4}cfu/ml and Actinomycetes count also ranged from 9.0×10^{-4}cfu/ml to 18.0×10^{-4}cfu/ml respectively.

Table 2: Mean separation of soil microorganism based on Growth performance in the Study Area

Site	Bacteria (cfu^{-1})	Fungi (cfu^{-1})	Actinomycetes (cfu^{-1})
High	33.0	40.4	14.8
Medium	54.8	50.6	126
Low	33	51.2	140
SE	8.03	9.48	18.7

Means with different letters(s) along the column are significantly different ($p \leq 0.05$).
Source: Field Survey, (2022)

1.1 Checklist of Micro Organisms in the Study Area

Table 3 shows the checklist of eleven (11) families containing seventeen (17) species of microorganisms identified in the study area. The families encountered were Actinomycetaceae (*Actinomyces crime*, *Actinomyces* yellow, *Actinomyces* blue), Aeromonadaceae (*Aeromonas* spp.), Trichocomacaceae (*Aspergillus niger*, *Aspergillus fumigatus*), Staphylococcaceae (*Staphylococcus aurus*), Streptococcaceae (*Streptococcus* spp.), Pseudomonadaceae (*Pseudomonas auroginosa*), Enterobacteriaceae (*Escherichia coli*, *Klebsiilla* spp.,` *Citrobacter* spp., and *Proteus* spp.), Bacillaceae (*Bacillus subtilis*, *Bacillus copus*), Lactobacillaceae (*Lactobacillus* spp.), Aeromonadaceae (*Aeromonas* spp.) and Streptomycetaceae (*Streptomyces* spp.) were present in the plantation soils..

The result in Table 4 shows the identified gram stained bacteria from nutrient agar plates which five 5 indicate gram negative and nineteen (19) show gram positive in term of shapes the result indicates fifteen (15) Cocci cluster, five (5) bacilli, and four (4) Cocci in chain respectively. Table 5 also shows the morphology of the bacteria in which four indicated flat and twenty raised respectively. The result in table 6 indicates the total fungal diluted in plates of potato dextrose agar, the pigmentation shows *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillin*, *Flavows*, *Microsponum canis*, *Microsponum andoumis*, Strestomycete and Actinomycetes respectively. The result in Table 7 shows the total Actinomycetes counts in diluted plates of starch casein agar, the pigmentation shows four cream colour, five cream pseudomonas species and fifteen cream yellow respectively.

Table 3: Checklist of Micro Organisms in the Study Area

S/N	Family	Species
1	Actinomycetaceae	*Actinomyces crime*
2	Actinomycetaceae	*Actinomyces yellow*
3	Actinomycetaceae	*Actinomyces blue*
4	Staphylococcaceae	*Staphylococcus aurus*
5	Streptococcaceae	*Streptococcus species*
6	Pseudomonadaceae	*Pseudomonas aeruginosa*
7	Enterobacteriaceae	*Escherichia coli*
8	Bacillaceae	*Bacillus subtilis*
9	Trichocomacaceae	*Aspargillus niger*
10	Lactobacillaceae	*Lactobacillus species*
11	Trichocomaceae	*Aspergillus fumigates*
12	Aeromonadaceae	*Aeromonas species*
13	Streptomycetaceae	*Streptomyces species*
14	Enterobacteriaceae	*Klebsiilla species*
15	Enterobacteriaceae	*Proteus species*
16	Enterobacteriaceae	*Citrobacter species*
17	Bacillaceae	*Bacillus copus*

Source: Field Survey, (2022).

DISCUSSION

Soil Micro - organisms (cfu-1) in the Study Areas

Findings of the soil microorganisms showed a wide diversity in the date palm plantation which were categorized based on high, medium and low performance sites.

These microorganisms (bacteria, fungi and actinomycetes) influence plant diversity and productivity according to van der Heijden et al. (2008). This is because they play important roles in the nutrient cycles and energy flows, providing essential services to the forest ecosystem. Soil fungi, for example have the function of catalyzing the turnover of complex organic resources, which can drive the degradation of organic matter. Bacteria generally utilize the easily available substrates decomposed by fungi. Conversely, they are affected by the plant communities as they depend on the products of plant photosynthesis: litter and rhizo-deposits (Wardle, 2006; Qiao et al., 2014; Prescott and Grayston, 2013). The microorganisms 'influence on plant diversity and productivity is in consonance with the findings of Lladó, et al. (2017) who stated that bacteria commonly harbor genes encoding plant cell wall-degrading enzymes and contribute significantly to the decomposition of organic matter. In addition, bacteria are the major natural agents responsible for N fixation in forest ecosystems and for other ecosystem processes, such as mineral weathering leading to the release of inorganic nutrients. The roles of bacteria and fungi, however, should not be viewed as separate. The high abundance of fungal biomass in forest

soils has multiple consequences for bacteria, including the creation of specific niches in the soil patches colonized by mycorrhizal fungi (i.e., the mycorrhizosphere) and soil mycelial mats, provision of nutrients via organic matter decomposition, and an increase in soil connectivity by fungal mycelia that allow certain bacteria to move across the environment.

TABLE 4: Identification of gram stained bacteria from nutrient agar plates (TBC).

S/NO	PLATE/SAMPLE	SLITE	GRAM REACTION	SHAPE
1	A	A	+VE	Cocci clustered
2	B	B	+VE	✓
3	C	C	+VE	✓
4	D	D	-VE	Bacilli
5	E	E	+VE	Cocci clustered
6	I	F	+VE	Cocci in chain
7	L	G	-VE	Bacilli
8	Q	H	+VE	Cocci clustered
9	U	I	+VE	✓
10	T	J	+VE	✓
11	R AND S	K	+VE	✓
12	J AND K	L	+VE	✓
13	X,W AND V	M	-VE	Bacilli
14	O,N,M AND P	N	+VE	Cocci clustered
15	F,G AND H	O	-VE	Bacilli
16		P	+VE	Cocci clustered
17		Q	+VE	✓
18		R	+VE	Cocci in chain
19		S	-VE	Bacilli
20		T	+VE	Cocci clustered
21		U	+VE	✓
22		V	+VE	✓
23		W	+VE	Cocci in chain
24		X	+VE	✓

Source: Field Survey, (2022).

TABLE 5: Total bacteria count/ml of 10-3 dilution plates of nutrient agar (TBC).

S/NO	PLATES	TOTAL BACTERIA COUNT	MORPHOLOGY
1	A	4.5×10^{-4} cfu/ml = 45 CFU/ML	Raised
2	B	3.0×10^{-4} cfu/ml = 30 CFU /ML	✓
3	C	3.7×10^{-4} cfu/ml =37 CFU /ML	✓
4	D	4.0×10^{-4} cfu/ml = 40 CFU /ML	✓
5	E	4.7×10^{-4} cfu/ml = 47 CFU /ML	✓
6	F	5.0×10^{-4} cfu/ml = 50 CFU /ML	✓
7	G	3.8×10^{-4} cfu/ml = 30 CFU/ML	✓
8	H	6.8×10^{-4} cfu/ml = 68 CFU/ML	✓
9	I	5.0×10^{-4} cfu/ml = 50 CFU/ML	✓
10	J	4.5×10^{-4} cfu/ml = 45 CFU/ML	✓
11	K	3.0×10^{-4} cfu/ml = 30 CFU/ML	✓
12	L	3.5×10^{-4} cfu/ml =35/CFU/ML	Flat
13	M	4.8×10^{-4} cfu/ml = 48 CFU/ML	✓
14	N	3.5×10^{-4} cfu/ml = 35 CFU/ML	✓
15	O	5.4×10^{-4} cfu/ml = 54 CFU/ML	✓
16	P	3.0×10^{-4} cfu/ml = 30 CFU/ML	Raised
17	Q	5.1×10^{-4} cfu/ml = 51 CFU/ML	✓
18	R	4.7×10^{-4} cfu/ml = 47 CFU/ML	✓
19	S	4.6×10^{-4} cfu/ml = 46 CFU /ML	✓
20	T	4.9×10^{-4} cfu/ml = 49 CFU/ML	✓
21	U	6.0×10^{-4} cfu/ml = 60 CFU/ML	✓
22	V	5.5×10^{-4} cfu/ml = 55 CFU/ML	✓
23	W	5.0×10^{-4} cfu/ml = 50 CFU/ML	✓
24	X	6.7×10^{-4} cfu/ml = 67 CFU/ML	✓

Source: Field Survey, (2022).
KEY: CFU = colony forming unit.
ML = mill liter.

Also, the fining shows that the numbers of bacterial communities decreases with decrease in depth. This agrees with the report of Lauber et al. 2009; Rousk et al. (2010) stating that bacterial abundance and diversity have been reported to decrease with decreasing soil pH. Similarly, the composition of fungal communities has been previously shown to differ substantially between litter and organic horizons, while deeper soil horizons showed greater similarity (O'Brien et al. 2009; Lindahl et al. 2007). In several forest types, this is due to the higher abundance of saprotrophic fungi in litter and the dominance of ectomycorrhizal species in deeper soil (Lindahl et al. 2007; Edwards et al. 2010).. Duran et al. (2019) stated that date palm can influence the composition and functioning of the soil bacterial community by altering the microclimate (via shading and through fall effects and uptake/transpiration of soil water), litter production, amount and quality of root exudates, and interactions with root symbiotic organisms such as mycorrhizal fungi.

This is because, in the forest ecosystem, trees can change the forest microclimate, and they can produce exudation from roots, litter, and wood debris; meanwhile, they (trees) interact with soil microbes and micro fauna through roots, and thus, can influence ecosystem properties. Date Palm can selectively attract and maintain rhizosphere microbes by root exudates, and at the same time, the microbial communities may strongly affect the growth of date palm by releasing mineral elements. Thus, the interaction between aboveground vegetation and soil microbial communities can influence the process of the forest ecosystem.

TABLE 6: Total fungal count/ml of 10-3 dilution plates of potato dextrose agar (TFC).

S/NO	PLATE	TOTAL FUNGAL COUNT/ML	PIGMENTATION
1	A	80.0× 10^{-4} cfu/ml = 800 CFU/ML	A. Nigera , flavons, A, Fumigatous
2	B	90.0× 10^{-4} cfu/ml = 900 CFU/ML	A, Flavows, parasiticum.
3	C	75.0× 10^{-4} cfu/ml = 750 CFU/ML	Microsporum spp, A. nigera, candid, panacea.
4	D	55.0× 10^{-4} cfu/ml = 550 CFU/ML	A.fumigatus, A , niger, and Flavows
5	E	12.00× 10^{-4} cfu/ml =1200 CFU/ML	Flavows , penicilline and A, niger
6	F	8.0× 10^{-4} cfu/ml = 80 CFU/ML	Fumigatum, penicillin , and A niger
7	G	68.0× 10^{-4} cfu/ml = 680 CFU/ML	Fumigatum, A niger
8	H	80.0× 10^{-4} cfu/ml = 800 CFU/ML	Flavows ,penicilline and microsponum canis
9	I	35.0× 10^{-4} cfu/ml = 350 CFU/ML	Penicilline, A. fumigatus, A , niger,
10	J	75.0× 10^{-4} cfu/ml = 750 CFU/ML	A, Flavows, Penicilline, A , niger,
11	K	12.50× 10^{-4} cfu/ml = 1250 CFU/ML	A, Flavows, A , niger, Penicillin,
12	L	13.10× 10^{-4} cfu/ml = 1310 CFU/ML	A, Flavows, A , niger, A.fumigatus,
13	M	91.0× 10^{-4} cfu/ml = 910 CFU/ML	A, Flavows, A , niger,
14	N	75.0× 10^{-4} cfu/ml = 750 CFU/ML	A, Flavows, A , niger, A.fumigatus,
15	O	83.5× 10^{-4} cfu/ml = 835 CFU/ML	A, Flavows, A , niger, A.fumigatus,
16	P	78.0 × 10^{-4} cfu/ml = 780 CFU/ML	A , niger, microsponum Andouini.
17	Q	61.0× 10^{-4} cfu/ml = 610 CFU/ML	A , niger, A, Flavows,.
18	R	70.0× 10^{-4} cfu/ml =700 CFU/ML	Penicillin, A, Flavows,
19	S	65.0× 10^{-4} cfu/ml = 650 CFU/ML	A , niger, A, Flavows, Fumigatum.
20	T	50.0× 10^{-4} cfu/ml = 500 CFU/ML	A , niger, A , Fumigatum
21	U	10.10× 10^{-4} cfu/ml =1010 CFU/ML	A , Fumigatum , A , niger,
22	V	12.80× 10^{-4} cfu/ml =1280 CFU/ML	A, Flavows, A , niger, strestomycete
23	W	7.80× 10^{-4} cfu/ml = 780 CFU/ML	A, Flavows, A , niger, strestomycete ,Actinomycetes
24	X	7.60× 10^{-4} cfu/ml =760 CFU/ML	A, Flavows, A , niger, penicillum, fumigation.

Source: Field Survey, (2022).
Key: CFU = colony forming unit.
ML= milliliter

TABLE 7: Total actinomycetes count /ml of 10-3 dilution plates of starch caiein agar (TAC).

S/NO	PLATES	TOTAL ACTINOMYCETES CONUT/ML	PIGMENTATION
1	A	2.0× 10^{-4} cfu/ml = 200 CFU/ML	Cream colour
2	B	1.9× 10^{-4} cfu/ml = 190 CFU/ML	✓
3	C	1.2× 10^{-4} cfu/ml = 120 CFU/ML	✓
4	D	1.6× 10^{-4} cfu/ml = 160 CFU /ML	✓
5	E	1.8× 10^{-4} cfu/ml = 180 CFU/ML	Cream pseudomonas spp
6	F	9.0× 10^{-4} cfu/ml = 90 CFU/ML	✓
7	G	6.0× 10^{-4} cfu/ml = 60 CFU/ML	✓
8	H	20.0× 10^{-4} cfu/ml = 200 CFU/ML	✓
9	I	7.0× 10^{-4} cfu/ml = 70 CFU /ML	✓
10	J	9.0× 10^{-4} cfu/ml = 90 CFU/ML	Cream yellow
11	K	21.0× 10^{-4} cfu/ml = 210 CFU/ML	✓
12	L	16.7 × 10^{-4} cfu/ml = 167 CFU/ML	✓
13	M	11.0× 10^{-4} cfu/ml = 110 CFU/ML	✓
14	N	22.0× 10^{-4} cfu/ml = 220 CFU/ML	✓
15	O	22.7× 10^{-4} cfu/ml = 227 CFU/ML	✓
16	P	19.0× 10^{-4} cfu/ml = 190 CFU/ML	✓
17	Q	15.0× 10^{-4} cfu/ml = 150 CFU/ML	✓
18	R	17.2× 10^{-4} cfu/ml = 172 CFU/ML	✓
19	S	21.2× 10^{-4} cfu/ml = 212 CFU/ML	✓
20	T	23.0× 10^{-4} cfu/ml = 230 CFU /ML	✓
21	U	24.5× 10^{-4} cfu/ml = 245 CFU/ML	✓
22	V	18.0× 10^{-4} cfu/ml = 180 CFU /ML	✓
23	W	24.0× 10^{-4} cfu/ml =240 CFU/ML	✓
24	X	21.0× 10^{-4} cfu/ml = 210CFU/ML	✓

Source: Field Survey, (2022).
Key: CFU = Colony forming unit.
ML = milliliter

Findings also show the presence of highly important bacterial and fungal groups (though in few numbers). In date palm plantation soils, microbial communities are affected by the changes in aboveground vegetation communities and soil environmental properties (such as nutrients, temperature, and moisture) influenced by human activity. These changes (as a result of agricultural activities) in the date palm plantation are believed to be responsible for the relatively low density of microorganisms in the study area. The loss of important microorganism as a result of application of pesticides in the plantation this also agreed with the findings of Mikayla et al. (2017) who stated that plantation could induce significant shifts in soil microbial community through biotic and abiotic factors, including species composition, above- and below-ground litter, and soil substrate quality and quantity, which are associated closely with soil microbial community.

CONCLUSION

Based on Based on the findings of this study, it can be concluded that; microorganisms were present in the study area. However, the numbers of microorganisms were very active in the date palm plantation soil. This is an indication that strong relationship exists between microorganisms and the date palm trees. The organism's breakdown complex organic matter, giving fertility to the soil, others like Actinomycetaceae through the ground, making the soil fertile and nutrients pass through and absorbed by the date palm trees. The date palm trees on the other hand produce food for microorganism through litters and exudates.

RECOMONDATION

The following recommendations are hereby made:

The Agricultural practices which combine trees and crops should be encouraged and application of agrochemicals is deleterious to the interrelationship of microorganisms and date palm plants. It also interferes with the varieties of food chains and food webs of the ecosystem.

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Research Article

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Survival and Growth Performance of Mahogany (*Swietenia macrophylla* King) Wildlings Using Biochar Soil Amendment

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Root length

ABSTRACT

The study was conducted to determine the effects of biochar application on the growth performance of mahogany wildlings. Four hundred eighty cuttings were used; treatments were arranged in Randomized Complete Block Design (RCDB), replicated 3 times and repeated three times. The study has four (4) treatments namely: T1 (Control), T2 (3:1 ratio of soil to biochar), T3 (2:3 ratio of biochar to soil) and T4 (2:2 ratio of biochar to soil). The study revealed that in terms of plant height, there is significant difference as referred to analysis of variance at 35 days after biochar application. T4 (mean of 23.81cm) is significantly higher than T1 (mean of 22.59cm) but not significantly different from T2 (mean of 23.33cm) and T3 (mean of 23.58cm). Analysis of variance revealed that there is no significant difference among treatment means in terms of diameter. At 70 days after biochar application, analysis of variance showed that there is significant difference in the number of leaves of mahogany wildlings i.e. treatment 4 (mean of 9.58) is significantly different from T1 (mean of 6.72), but not significantly different from T2 (mean of 7.68) and T3 (mean 8.47). Based on the analysis of variance in terms of root length, there is no difference among means. In terms of survival, there is no significant difference among treatment means. All treatments obtained a mean of 100% which proved that all treatments are comparable to each other.

The result concludes that application of biochar as soil amendment did not affect the mahogany wildlings in terms of plant diameter, root length, and survival. However, in terms of plant height and number of leaves, the application of biochar showed significant effects on mahogany wildlings. It was also revealed that application of biochar at 2:2 ratio of biochar to soil performed better than other treatments.

INTRODUCTION

Mahogany (*Swietenia macrophylla* King) is a member of the family Meliaceae, and is one of the best-known and more frequently used tree species of forest stands. Due to its biological and commercial characteristics, mahogany has a large potential to become the basis for a sustainable use and management system of the tropical forest, applied in the framework of appropriate silvicultural practices. Genetic diversity in the tropical forests, where the mahogany grows, is rapidly decreasing due among other reasons to deforestation processes and natural population fragmentation. The first phenomenon reduces the population size and natural communities' fragmentation; it makes the gene exchange difficult and may isolate continuous populations of a given species until its genetic diversity is lost, as a result of endogamy and genetic erosion. These phenomena highlight the

enormous risks that tropical forest resources face, especially some species of commercial value such as mahogany, therefore justifying the urgent need to better understand genetic diversity at its different levels and use such knowledge in the management, improvement, and conservation practices of those important genetic forest resources (Kumar, 2015).

Soil is the most important source and an abode for many nutrients and microflora. Due to the rapid depletion of agricultural areas and soil quality by means of an ever-increasing population and excessive addition of chemical fertilizers, rehabilitated attention is a need of the hour to maintain sustainable approaches in agricultural crop production (Jyotiet al. 2019).

One of the traditional ways to improve the energy efficiency of agricultural systems is to the soil part of the biomass produced by crops, which in many cases is removed from the field to be used for other purposes or even destroyed. Returning crop waste into the soil, either without any processing or through organic amendments represents a management strategy that, in addition to the improvement of energy efficiency for agro-ecosystems, may help to combat soil degradation phenomena. Soil amendment includes all inorganic and organic substances mixed into the soil for achieving a better soil constitution regarding plant productivity. Soil amendment does not include mulching, which include substances lying on top of the soil. The reason for soil amendment is to provide a better environment for roots and plant growth: this includes the improvement of the soil structure and water holding capacity, the availability of nutrients, and the living conditions for soil organisms which are important for the plants to grow. Furthermore, a better soil texture and better root growth avoid soil degradation during heavy rains or in windy regions. It also supports the nutrient cycle when organic amendments are used (e.g. manure). Of course, it is also very important that a crop is planted which is suitable for the given climate. Basically, any organic or inorganic material that is added to the soil and improves its quality can be considered a soil amendment (West Coast Seeds, 2011).

An alternative to returning biomass into the soil is the integration of biochar as an organic amendment in the crop production process. The term biochar can be defined as a carbonaceous material obtained from biomass by thermal decomposition at low or no oxygen concentration, through a thermo-chemical process known as pyrolysis. There is also consensus that its specific application to soil is expected to sustainably sequester carbon and improve soil functions (Lehmann et al. 2021).

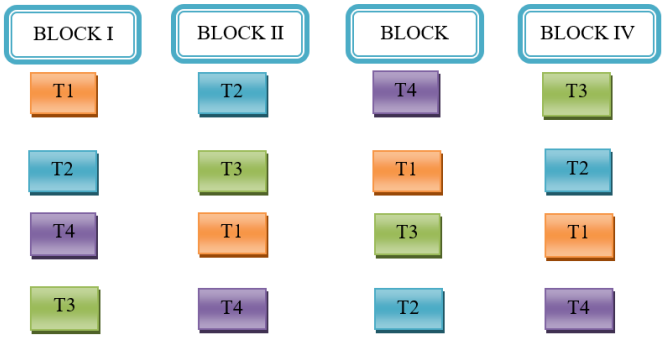
Therefore, the main objective of the study was to determine the effect of biochar application in potted mahogany wildlings after transplanting.

MATERIALS AND METHODS

Experimental Design and Treatment

Treatments were arranged in Randomized Complete Block Design (RCDB). Thirty (30) wildlings per treatment will be used and replicated thrice. A total of four hundred eighty (480) mahogany wildlings were used in this experiment.

Experimental Layout



LEGEND:

T1 = Control
T2 = 3:1 ratio of soil to biochar
T3 = 2:3 ratio of biochar to soil
T4 = 2:2 ratio of biochar to soil

Source of Biochar

The biochar that was used in this research is commercial charcoal obtained from agricultural (rice hulls) wastes through a pyrolysis process provided by Sambali Beach Farm in Pampanga, Philippines. Sambali Beach Farm is founded in 2000 by Ching Camara, the farm is at the forefront of Biochar in the Philippines, and it is the official Biochar-making center accredited by the Philippine Biochar Association (PBiA).

Nutrient Content of Biochar

Department of Agriculture, Regional Soils Laboratory of San Fernando, Pampanga stated that the biochar was analyzed and tested as fertilizer and the usual parameters tested are nitrogen (N), phosphorus (P), and potassium (K). According to the study of Purakayastha et al. (2019), biochar can supply nutrients such as nitrogen (N),

phosphorus (P), potassium (K), and other trace elements inherently present in the original feedstock used for biochar production.

Constructing the Nursery Building

The researcher first leveled the soil to make it even to the ground, and then a suitable area was measured to use in experiments. The mainframe of the site is bamboo. Roof and fence materials are also bamboo panels that are covered with a garden net. The researcher also used coconut or banana leaves to cover the roof so that some sunlight can still pass through, these will help to support the plants during their recovery period.

Pot preparation

Garden soil and its incorporation into biochar is the substrate used in this research. The soil media were sterilized for 40 minutes. Afterward, it was mixed manually into biochar in their corresponding volume before placing in pots wherein 8" x 8" size of polyethylene bags were used as experimental pots.

Wildlings Preparation

Mahogany wildlings were collected from the Mahogany Forest of Mount Arayat National Park (now Mount Arayat Protected Landscape), Ayala, Magalang, Pampanga. The researcher chose wildlings that are healthy and free from diseases.

Transplanting of Wildlings

The wildlings were transplanted in prepared polyethylene bags. Leafages of the mahogany wildlings were cut to retain one-third to one-half of the original length to reduce transpiration and the plants were placed in a partially shaded area. In twenty (25) days after transplanting the initial data was gathered.

Maintenance of the Experimental Plot

The area of the study was monitored to keep the plants safe. Manual removal of weeds in the plants was conducted every day. To maintain the moisture of the mahogany wildlings, watering equally was done as needed.

Data Gathering Procedure

The gathering of data was conducted every 7 days for 35 days of the duration of the study. The following parameters were gathered:

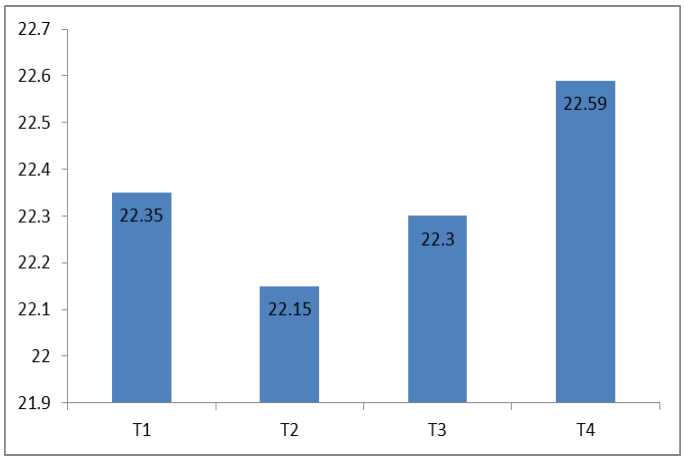
1. Plant height Increment. The height of plants was measured 25 days after transplanting (initial), then the following was measured every 7 days for 35 days after biochar application (DABA). Plants will measure using tape measured 2 cm above the base up to the highest apex of the plant.
2. Stem Diameter Increment. The diameters of plants were measured 25 days after transplanting (initial), and then the following was measured every 7 days for 35 DABA. Plants were measured using a digital calliper 2cm above the base of the plant. A permanent mark was the basis for measuring the plant diameter.
3. Number of Leaves. Leaves were counted and marked with permanent marker 25 days after transplanting (initial), and the leaves produced and visible on the plant, including the tips of new leaves just beginning to emerge were counted every 7 days at 35 DABA.
4. Length of roots in (cm) - determine the length of roots of mahogany wildlings at 35 DABA. The roots were measured from the base up to the root apex using a tape measure;
5. Percent of Survival. Determine the percent of survival of all the treated mahogany seedlings at 35 DABA.

Statistical Analysis

The data gathered in this study were statistically analyzed using one-way analysis of variance (ANOVA) in Randomized Complete Block Design (RCBD) to determine if there are significant differences among the parameter tested. Further, posthoc tests using LSD were carried out to identify specific treatments that bear significant differences.

RESULTS AND DISCUSSION

Figure 1 showed the initial plant height of the mahogany wildlings. Treatment 1 measures 22.35cm, T2 22.15cm, T3 22.3 and T4 measures 22.59cm.

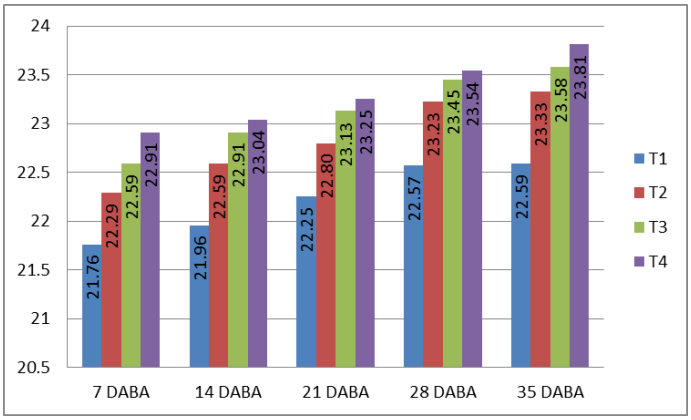


T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 1. Initial plant height at 25 days after transplanting

Seven (7) days after biochar application highest mean was recorded under T4 obtained a mean of 22.91cm followed by T3 a mean of 22.59cm, T2 a mean of 22.29cm, and the lowest mean was obtained at T1 a mean of 21.76cm. Fourteen (14) days after biochar application highest mean was recorded under T4 obtained a mean of 23.04cm followed by T3 a mean of 22.91cm, T2 a mean of 22.59cm, and the lowest mean was obtained at T1 a mean of 21.96cm. Twenty-one (21) days after biochar application highest mean was recorded under T4 obtained a mean of 23.25cm followed by T3 a mean of 23.13cm, T2 a mean of 22.80cm, and the lowest mean was obtained at T1 a mean of 22.25cm. 28 DABA highest mean was recorded under T4 obtained with a mean of 23.54cm followed by T3 with a mean of 23.45cm, T2 with a mean of 23.23cm, and the lowest mean was obtained at T1 with a mean of 22.57cm, and 35 DABA's highest mean was recorded under T4 obtained with a mean of 23.81cm followed by T3 with a mean of 23.58cm, T2 with a mean of 23.33cm, and the lowest mean was obtained at T1 with a mean of 22.59 cm.

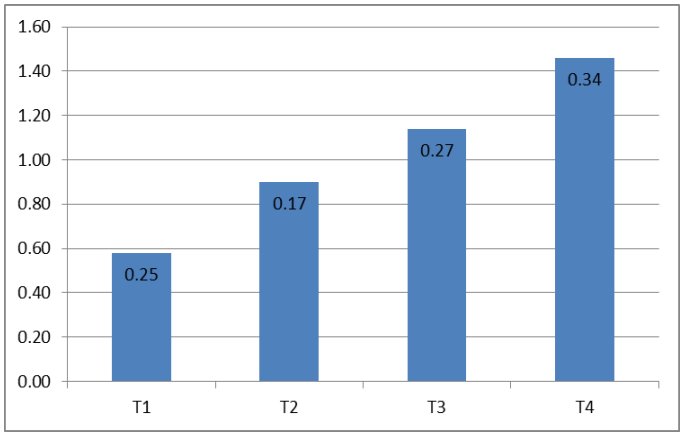
Figure 2 showed the calculated mean, in the plant height of mahogany wildlings. The analysis of variance shows that there is a significant difference ($P<0.05$) in terms of height across treatments at 35 days after biochar application. The LSD analysis revealed that T4 obtained a mean of 23.81cm is significantly higher than T1 a mean of 22.59cm but not significantly different from T2 a mean of 23.33cm and T3 a mean of 23.58cm.



T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 2. Mean plant total height (cm)

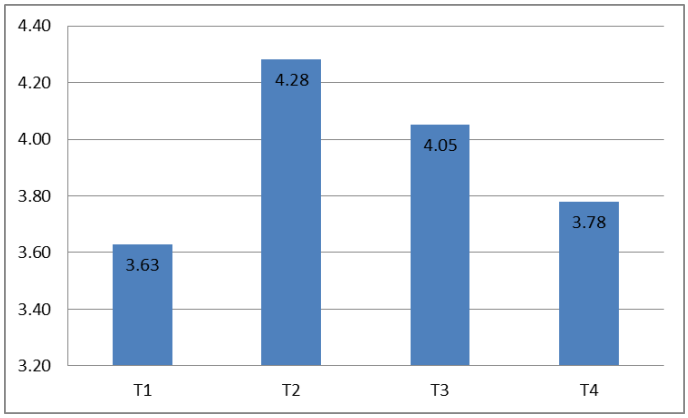
Biochar has been shown to promote plant productivity and yield through several mechanisms. Physical conditions change with biochar, its dark color alters thermal allowing more time for growth compared with controls (Beiderman and Harpole, 2012). The biochar-induces improvement in soil water holding capacity and soil nitrogen or phosphorus availability enhancing plant productivity. The increase in soil alkalinity following biochar amendment could also be beneficial to plant growth, which was further supported by our findings indicating an increase in biochar Ph (Yu. et al. 2018).



T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 3. Mean plant height (cm) increment

Plant height increment was calculated as shown in Figure 3, T1 obtained a mean of 0.25cm, followed by T2 with 0.17cm, T3 measures 0.27cm, and T4 with 0.34cm which was recorded to be the highest increment obtained in the wildlings. This showed that T4 is significantly higher than T1, but not significantly different from T2 and T3 as referred to analysis of variance.



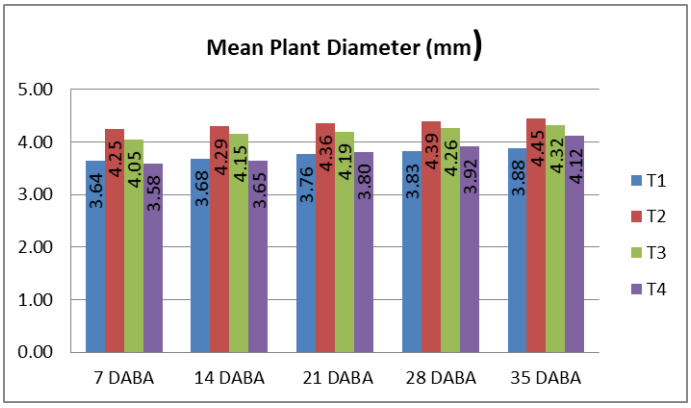
T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 4. Initial plant stem diameter (mm)

The initial plant stem diameter (mm) was also measured as shown at Figure 4 treatment 1 measures 3.63mm, T2 4.28mm, 4.05mm, T3 4.05mm and 3.78mm.Figure 5 showed that at seven (7) to thirty- five (35) days after biochar application analysis of variance revealed that there is no significant difference among treatment means. Seven (7) days after biochar application highest

mean was recorded under T2 obtained a mean of 4.25mm, followed by T3 a mean of 4.05mm, T1 a mean of 3.64mm, and the lowest was obtained at T4 a mean of 3.58mm. 14 DABA highest mean was recorded under T2 obtained with a mean of 4.29 mm, followed by T3 with a mean of 4.15mm, T1 with a mean of 3.68mm, and the lowest was obtained at T4 with a mean of 3.65mm. 21 DABA highest mean was recorded under T2 obtained with a mean of 4.36 mm, followed by T3 with a mean of 4.19 mm, T4 a mean of 3.80 mm, and the lowest was obtained at T1 with a mean of 3.76mm. 28 DABA highest mean was recorded under T2 obtained at a mean of 4.39mm, followed by T3 with a mean of 4.26mm, T4 with a mean of 3.92mm, and the lowest was obtained at T1 with a mean of 3.83 mm and 35 DABA highest mean was recorded under T2 obtained a mean of 4.45 mm, followed by T3 a mean of 4.32mm, T4 a mean of 4.12 mm, and the lowest was obtained at T1 a mean of 3.88mm.

These were revealed to be comparable to each other, although there is an observed numerical difference between the highest and lowest mean. This indicates that the wildlings used were almost homogenous in diameter.



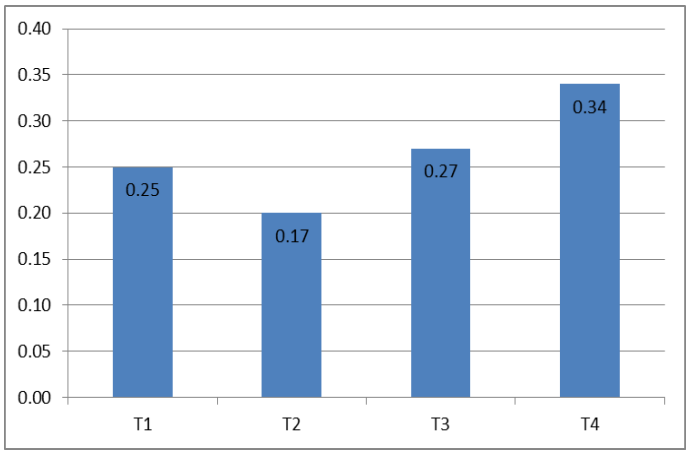
T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 5.Stem diameter (mm)

This chart shows that the growth of the wildlings is correlated to their mortality rate in this study. Though the results state that they didn't have any significant difference, they still projected a numerical significance as they have a consistent increase from 7 to 35 days after biochar application even if it's relatively low. If they continue to show growth at this pace, despite how low, definitely, they would still thrive.

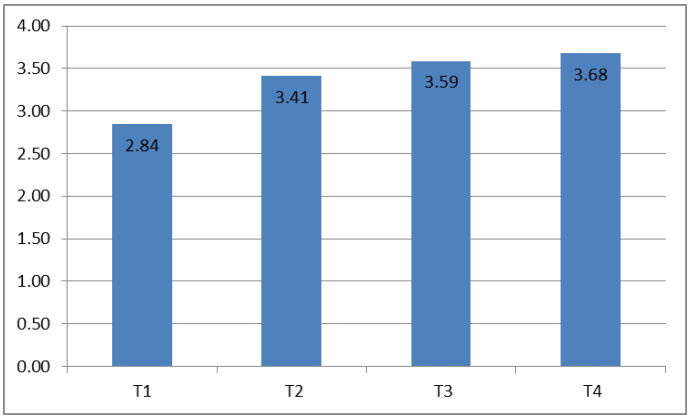
Plant diameter was also calculated for increment as illustrated in Figure 6 T1 obtained a mean diameter increment of 0.25mm, T2 of 0.17mm, T3 with 0.27mm, and T4 with 0.34mm. All treatments are comparable in terms of plant diameter as reflected in the analysis of variance which reveals no significant difference. The lower stem diameter growth indicates that these plants had a reduced rate of photosynthesis (Kozlowski and Pallardy, 1997).

In general, biochar amendment increases soil nitrogen availability and retention; improves soil water-holding capacity, increases soil pH and action exchange capacity, decreases soil bulk density, facilitates beneficial microorganisms, and limits the bioavailability of heavy metals, which are associated with increases in plant photosynthesis. In addition, biochar amendment and the induced changes in soil properties can also affect plant performance by altering growth and traits (Chen et al. 2019).



T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 6. Stem diameter (mm) increment

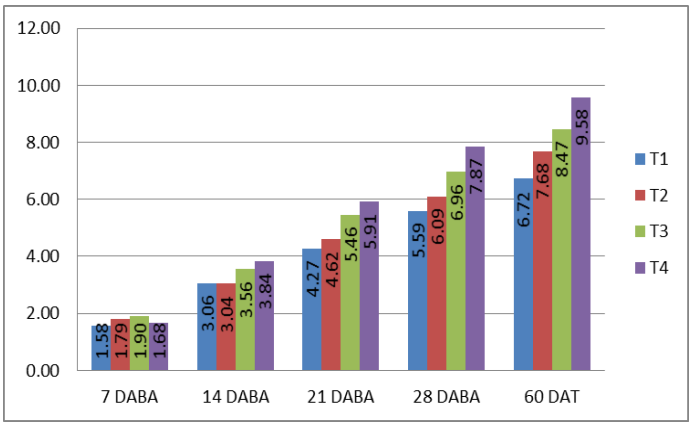


T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 7. Initial number of plant leaves

The initial number of plant leaves was also measured as shown in Figure 7 treatment 1 measured 2.84, T2 3.41, T3 3.59, and T4 measured 3.68.

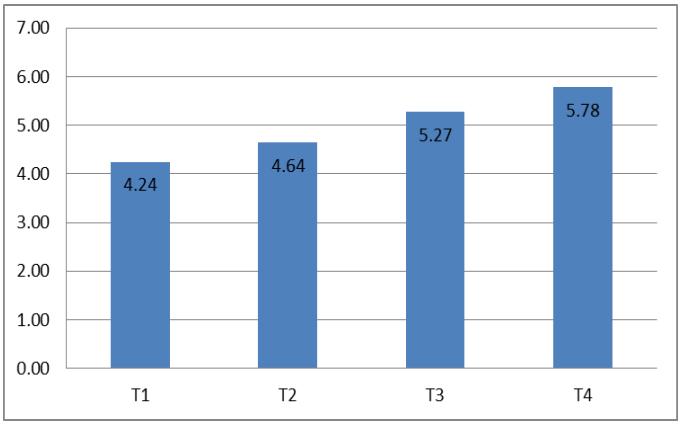
Figure 8 showed at thirty-five (35) days after biochar application, analysis of variance showed that there is a significant difference (P<0.05) across treatments. The LSD analysis revealed that the number of leaves in treatment 4 obtained a mean of 9.58 is significantly different from T1 a mean of 6.72, but not significantly different from T2 a mean of 7.68, and T3 a mean of 8.47.



T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 8. Number of new leaves

As shown in Figure 8, number of new leaves was observed significantly at the higher application of biochar. When biochar is applied to the soil, it comes in close contact with the plant root and has a direct effect on root growth, thereby affecting root morphology, which in turn has a profound impact on the growth of the plant shoot (Q. Zhu et al., 2018).

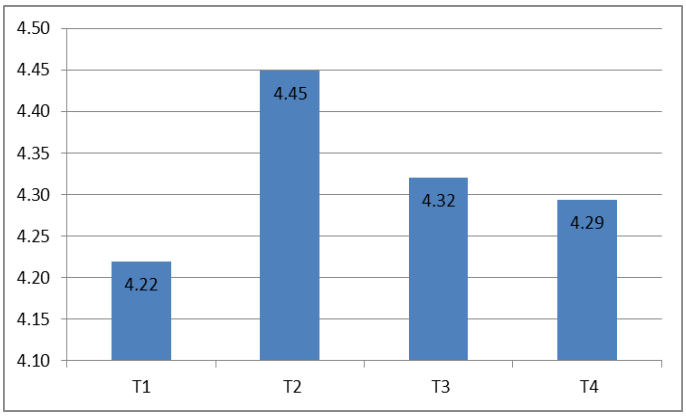


T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 9. Added Leaves

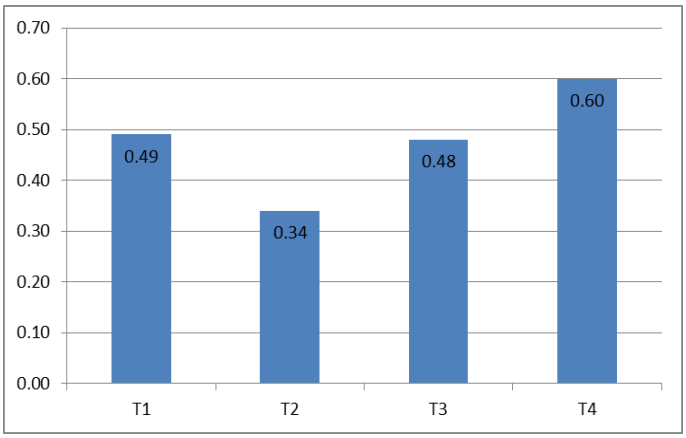
Added leaves were also calculated as illustrated in Figure 9 wherein T1 obtained a mean of 4.24, T2 with a mean of 4.64, T3 with a mean of 5.27, and T4 with 5.78. It was revealed that there are significant differences in terms of the number of leaves as referred to in the analysis of variance.

The initial root length of the plants shown in Figure 10 treatment 1 measured 4.22cm, T2 4.45cm, T3 4.32cm, and T4 measures 4.29cm. Based on the analysis of variance, the difference among means is not significant. This showed that all treatments applied to mahogany wildlings have no effects in terms of root length. This indicates that the root length of the wildlings used was almost uniform.



T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 10. Initial root length (cm)



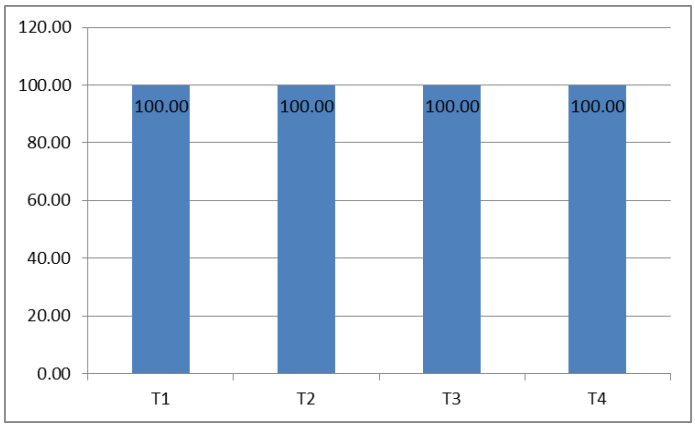
T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 11. Root length increment (cm)

When biochar is applied to the soil, it comes in close contact with the plant root and has a direct effect on root growth, thereby affecting root morphology, which in turn has a profound impact on the growth of the plant shoot (Q. Zhu et al. 2018).

Analysis of variance revealed that there is no significant difference among treatment means. All treatments

obtained a mean of 100 % which proved that all treatments are comparable to each other.



T1 = Control, T2 = 3:1 ratio of soil to biochar, T3 = 2:3 ratio of biochar to soil, T4 = 2:2 ratio of biochar to soil

Figure 12. Survival rate (%)

Summary of Findings

The study was conducted from May to July 2021in the yard of the David Family located at San Vicente, Magalang, Pampanga to determine the effects of different applications of biochar on the survival and growth performance of mahogany wildlings. Three hundred sixty wildlings were used. Treatments were arranged in Randomized Complete Block Design (RCDB). The study has four (4) treatments namely; T1 (Control), T2 (3:1 ratio of soil to biochar), T3 (2:3 ratio of biochar to soil), and T4 (2:2 ratio of biochar to soil). The treatments were replicated three times.

The study revealed that in terms of plant height, there is a significant difference as referred to the analysis of variance 35 days after biochar application. T4 obtained a mean of 23.81cm is significantly higher than T1 a mean of 22.59cm but not significantly different from T2 a mean of 23.33cm and T3 a mean of 23.58cm. Analysis of variance revealed that there is no significant difference among treatment means in terms of diameter. T1 obtained 3.88mm, T2 mean of 4.45mm, T3 a mean of 4.32mm, and T4 a mean of 4.12mm.At seventy 35 days after biochar application, analysis of variance showed that there is significant difference in the number of leaves of mahogany wildlings. Treatment 4 obtained a mean of 9.58 is significantly different from T1 a mean of 6.72, but not significantly different from T2 a mean of 7.68, and T3 a mean of 8.47. Based on the analysis of variance in terms of root length, it was revealed that there is no difference among means. T1 obtained 0.49cm, T2 mean of 0.34cm, T3 a mean of 0.48cm, and T4 a mean of 0.60cm.In terms of survival rate analysis of variance revealed that there is no significant difference among treatment means. All treatments obtained a mean of 100 % which proved that all treatments are comparable to each other.

CONCLUSION

Based on the result of the study, the application of biochar as soil amendment did not affect the mahogany wildlings in terms of plant diameter, root length, and survival. However, in terms of plant height and the number of leaves, the application of biochar showed significant effect on mahogany wildlings. It was also revealed that application of biochar at 2:2 (ratio of biochar to soil) performed better at other treatments.

For further study, the researcher recommends the following:

- Incorporate the use of other fertilizers with Biochar;
- Incorporate the use of Biochar in improving the growth of other species;
- Measure other parameters using other factors such as watering frequency; and
- Follow the prescribed data gathering period or even prolong it for better reliable results.

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DOCUMENTATIONS

1. Overview of the Study

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B. 35 days after biochar application

3. Nursery set up

4. Soil Sterilization

5. Transplanting of Mahogany wildlings

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Effect of De-Worming Drugs for Intestinal Parasites Control on Laboratory Rats (*Rattus norvegicus*) Breeding in DMR

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ABSTRACT

Pinworms remain most prevalent in laboratory mice and rats. Rats are usually infected with *Syphacia muris* and mice with *Syphacia obvelata* and *Aspiculuris tetraptera*. Therefore, the study was conducted to eliminate worm infection using deworming drugs on laboratory rats in DMR during the study period from March 2021 to February 2022. In of rats the present study, thirty males and thirty females of Wistar rats (*Rattus norvegicus*) from DMR were randomly selected and 10 each were housed in cages separately according to the treatment of three deworming drugs as fenbendazole, albendazole and parazivet groups of rats. Before the treatment of deworming drugs, all groups of rats were detected worm parasite's eggs by the method of taping (Graham). Out of 60 rats, 55(91.67%) rats were positive for both Pinworm (*Syphacia muris*) and Ascariasis (*Ascaris suum*) eggs. Of these, the highest density of mixed infection was found 40 (72.73%) (Pinworm + Ascariasis) followed by Pinworms only 8(14.55%) of rats and lowest positivity was observed Ascaris suum eggs only 7(12.73%) rats respectively. Among them 28 (93.33%) samples of males and 27 (90%) samples of females were found parasite positive. After treatment of deworming drugs, 100% reduction was observed in all groups of rats. All the deworming drugs were found very effective to control the Pin worm and Ascariasis infection. To reduce reinfection, monthly treatment is needed to control or eliminate the worm infection in laboratory animals.

INTRODUCTION

Pinworms are most prevalent in laboratory mice and rats (Clifford and Cosentino 006a,b; Livingston and Riley 2003). Rats are usually infected with *Syphacia muris* and mice are infected with *Syphacia obvelata* and *Aspiculuris tetraptera* (Baker, 2007). Rats can be incidental hosts of *Syphacia obvelata* and mice can be incidental hosts of *Syphacia muris* (Baker, 2007, Phillipson, 1974). And also rats are infected with *Aspiculuris tetraptera* (Mathies, 1959). In the late 1980s and early 1990s, some viruses prevalent in laboratory mice such as Sendai virus, mouse hepatitis virus (MHV), epizootic diarrhea of infant mice (EDIM), and the only parvovirus and helminths are prevalent in laboratory mice and rats. In rats, the picture was similar, with prevalent of viruses including the Sendai virus, coronavirus (also called sialodacryoadenitis virus, SDAV), and the rat parvoviruses. In addition to the viruses, pinworms were prevalent in both mice and rats (Casebolt et al. 1988; Jacoby and Lindsey, 1998; Lussier and Descoteaux, 1986). Pinworms are nematode

parasites (Family Oxyuridae) that have simple, direct life cycles and are frequent contaminants of both specific pathogen free (SPF) and conventional colonies of laboratory rats and mice.

Pinworms are transmitted through the ingestion of embryonated eggs. Two species of pinworms *Syphacia obvelata* and *Aspiculuris tetraptera* commonly infect laboratory rats and mice. The prevalence of pinworms in an infected rodent population depends on many factors, including parasite environmental load, gender, age, strain, and immune status. Males tend to have higher burdens than females, while young animals tend to have higher worm burdens than older animals.

Mice and rats are the most common laboratory animals used in research and testing of many kinds of traditional and pharmaceutical research. The parasite infections can affect investigations by inducing physiological and immunological alterations in the hosts, increasing or diminishing host susceptibility to experimental stress, inducing tissue damage, stimulating abnormal tissue growth, competing with the host for nutrients, decreasing the volume of the host's blood and body fluids and by mechanical interference (Baker, 2007). Still little is known about the effects of environmental changes on the biological variation in experimental results (Stahl 1963). In the mouse and rat caecum and colon, *Qspicularis tetraptera* may be found together with *Syphacia muris* or *Syphacia abvelata* (Taffs, 1979).

Syphacia muris is the most prevalent pinworm of rats. The life cycle of *Syphacia muris* is direct and completed in 7 to 8 days, (Lewis and Silvq, 1986) making this particular pinworm ideal for epidemiologic study. Adult worms of *Syphacia muris* inhabit the cecum and colon, and female worms migrate to the anus and deposit all their eggs on the perianal region of the host before dying. Within a few hours, the eggs developed into embryonic and they are considered infective (Stahl, 1963). Infection is believed to occur via 3 modes: (1) direct ingestion of the eggs; (2) ingestion of food or water contaminated with the eggs; and (3) retro infection (Chan 1952). Ingestion of eggs is considered to be the primary mode of infection, and the eggs are reported to remain viable within the environment for as long as 4 weeks (Cliford and Watson, 2004, Dix et al. 2004). Antemortem diagnosis traditionally is made by identification of these eggs on a perianal cellophane tape, given the ease of collection and interpretation, although direct examination of the cecum postmortem is considered the most dependable method for *Syphacia muris* diagnosis (Anya, 1966).

Ascariasis prevalence was found in humans and animals Kindem. *Ascaris suum* is mostly infected to rats, mice, swine and pigs. *Ascaris suum* infection is established orally by third stage larvae after their development from embryonated eggs. The third stage larvae invade the small intestine of host migrate into the lever and lung, and finally reach to the cecum and/or proximal colon, where they develop into adult worms (Tsuji et al. 2003). Ethiopian Study revealed that the prevalence of helminthiasis was higher in mice (28.57% than in rats (7.41%) (Derothe et al. 1997). Internal and external parasites remain a significant concern in laboratory rodent facilities, and many research facilities harbor some parasitized animals (Institute for Laboratory Animal Research, 2011). This study reported that the data on the presence of helminth parasites, mainly with regard to pinworm species in laboratory rat colonies. At the same time, to fine out the effectiveness of deworming drugs on helminth parasites in laboratory rats colonies of DMR. Therefore, the objective of the study was to fine out the effectiveness of deworming drugs for intestinal parasites control on laboratory rats (*Rattus norvegicus*) breeding in DMR.

MATERIALS AND METHODS

Study Area

The study was conducted on laboratory rats in the Laboratory Animal Service, Department of Medical Research, Ministry of Health, Yangon, Myanmar.

Study period

The study period was one year, from April 2021 to March 2022.

Study Design

Laboratory based descriptive study design was used.

METHODOLOGY

Thirty males and thirty females of Wistar rats (*Rattus norvegicus*) from DMR were randomly selected. Laboratory rats were housed in cages (250 mm x 170 mm x 100 mm) of their breeding rooms separately. The temperature and humidity of the experimental room was maintained at 22±2°C and humidity 80-90%. An exhaust fan and air-conditioner were provided for good air ventilation in room. Selected rats were given to free access the diet and tap–water. Before the experiment, 10 Male and 10 female each of rats were separated into 6 different cages (3cages for male and 3 cages for female). Cages were labeled according to three deworming drugs

(fenbendazole, albendazole and parazivet) respectively and then all the rats were detected pin worms eggs using taping method (Graham, 1941), after that each group of rats was treated with deworming drugs according to treatment regimens for rats and mice were described in the reference literature. For rats, treatment generally involves a week-off feeding containing fenbendazole.

Pinworm eggs are resistant to desiccation and many common disinfectants, but are susceptible to high temperatures. In this experimental study, different deworming drugs (fenbendazole, albendazole and parazivet) were applied to selected groups of rats. According to the instruction of therapy, it was done by oral administration of 10mg/kg/day for 10 consecutive days. After 7 days of treatment, all groups of rats are checked, and detected the Pinworm and Ascariasis parasite's eggs again by the method of taping (Graham, 1941).

Analysis of Data

The mean and standard deviation of each parameter were calculated by standard statistical methods. The positive rate was calculated in percent.

RESULTS

Table 1. Shows that before the treatment of deworming drug in the rat population, the positivity rate of eggs was found, in 55(91.67%) out of 60 rats for both Pinworm (*Syphacia muris*) and *Ascaris suum* eggs. Of these, the highest density of mixed infection (Pinworm + Ascariasis) was found in 40 rats = (21male +19 females) (72.73 %) in the rat's population, followed by (14.55 %) of only pinworms (*Syphacia muris*) positivity 8 (3 males + 5 females), and lowest positivity was observed 7 (4 male + 3 Female) (12.73 %) of only *Ascaris suum* eggs.

Table 1. Positivity rate of Pinworm and Ascariasis eggs, before and after treatment of deworming drugs in laboratory-reared Wistar rats (*Rattus norvegicus*) from DMR

Rattus norvegicus	**No. of Sample**	**Treatment of drugs**	**Before treatment**			**Total positive**	**After treatment**			
			Pw eggs +ve (*Syphacia muris*)	***Ascaris suums* eggs +ve (*A.*)**	**Mixed (Pw+Ascariasis)**		**Pw Eggs +ve**	**Ascar eggs +ve**	**Mixed**	**% Reduction**
Rats (Males)	10	FBZ	2	1	7	10	0	0	0	100
	10	ABZ	0	2	6	8	0	0	0	100
	10	PZV	1	1	8	10	0	0	0	100
Rats (Female)	10	FBZ	2	2	5	9	0	0	0	100
	10	ABZ	2	0	8	10	0	0	0	100
	10	PZV	1	1	6	8	0	0	0	100
Total	60		8 (14.55%)	7 (12.73%)	40 (72.73%)	55	0	0	0	100
Total + ve	60		55 (91.67%)							

FBZ= Fenbendazole, ABZ=Albendazole, PZV = Parazivet, Pw=Pin worm, Ascar=Ascariasis, +ve=positivity

A total of 30 males and 30 females were tested for intestinal worms parasites before deworming drugs administration, 28 (93.33%) samples of males and 27 (90%) samples of females were found worm parasite positive. Of these 10 each of the rats was positive for pinworm and ascariasis eggs of PZV Parazivet and FBZ Fenbendazole group. And 8 rats were found positive for both parasite aggs in ABZ Albendazole group. In female groups 10 rats were positive in ABZ Albendazole and 9 and 8 female rats were positive for pinworm and ascariasis in FBS and PZV groups. After treatment of different deworming drugs as Fenbendazole, Albendazole, Parazivet, on rats, all the rats were found parasite eggs negative and a 100% reduction was observed in all groups of rats.

DISCUSSION

Mice and rats are very useful animals in different kinds of research in the laboratory. Some are useful for cancer research, some are useful for snake bite research, some are useful for bone healing research and some are useful for toxicity research. Therefore, healthy laboratory animals are needed to access accurate and good results. In the present study before the treatment deworming drug laboratory rats were found pinworm (*Syphacia muris*) and *Ascaris suum* eggs positive by the examination of the tapping method under the compound microscope with 40X lance. A total of 60 laboratory rats (30 male + 30 Female) were examined 28 male and 27 female rats

were positive for pinworm and ascariasis eggs. Of these pinworm and ascariasis mixed positivity was found higher than individual parasite eggs positivity in both male and female rats. In the male group 21 rats were infested with mixed (pin worm + Ascariasis) infection, only 3 were pinworm eggs and 4 were Ascariasis eggs positive. Similar trim of infection results as male has been found in female groups. In female groups 19 mixed positivity of pinworm and ascariasis eggs and 5 was pinworm and 3 were ascariasis eggs positive individually and finding observed that male rats have a higher burden of infection than female rats. It may be due to pinworm infestations continuing because of prolonged infections, inefficient diagnosis, and the survivability of eggs of some species in the environment. Other researchers revealed that the prevalence of pinworms in an infected rodent population depends on many factors, including environmental load, gender, age, strain, and immune status. Males tend to have higher parasite burdens than females, while young animals tend to have higher worm burdens than older animals. Laboratory mice tend to be more resistant to experimentally induced infection than wild mice. Athymic mice, as might be expected, have an increased susceptibility to infection (Meade. and Watson, 2014). In the present study, rats were infected with *Ascaris suum* in high density, which is morphologically different from *Ascaris lumbricoides* (Maung, 1973). Recent studies have revealed that *Ascaris suum* of swine origin can develop in humans, indicating its zoonotic importance (Anderson et al. 1993; Peng et al. 1988). Although numerous studies have been carried out thus far to characterize the two species of parasites on a morphological basis, species discrimination between *Ascaris lumbricoides* and *Ascaris suum* has been controversial (38 Abebe et al. 2002; Kurimoto, 1974; Maung, 1973; Nielsen et al. 1997).

After being treated with different deworming drugs (fenbendazole, albendazole and parazivet) in rat groups, all the parasite eggs were disappeared or negative in three tested groups of rats by the diagnosis of the taping method. All the rats were free from pinworm and Ascariasis eggs after treatment of 7 days. Meade and Watson (2014) revealed that egg hatching after treatment with chlorine dioxide was significantly reduced as compared with that of unexposed control eggs ($P < 0.01$). Eggs exposed to 400 mg/L chlorine dioxide gas hatched at a rate of 0.3%. Biologic indicators supported efficacy of the gaseous treatment. Furthermore, these eggs showed morphologic differences in the appearance of the capsule, as compared with control eggs. Liquid forms were significantly ($P < 0.01$) less effective at preventing hatching than the gaseous form of chlorine dioxide. On the basis of his data, he recommended that perianal tape testing should occur as close as possible to the peak egg-shedding time of 1400, to maximize the sensitivity of this particular diagnostic test (Meade and Warson, 2014).

In the present study, all the infected rats were found free from pinworm and ascariasis eggs after treatment with deworming drugs, although other researchers informed that Eggs may contaminate ventilation ducts (Hoag, 1961) or shared equipment or procedure areas (Huerkamp, 1993) and can recontaminate a colony after the completion of treatment. Knowledge of egg longevity in the environment is important to determine the need for environmental decontamination, but specific data are unavailable. Aspiculuris tetraptera eggs are thought to be long-lived in the environment, remaining dormant for several months at 4°C Stahl 1966). Anya (1966a) reported, however, that culturing newly shed eggs at 37°C accelerated embryonation, decreased the number of viable eggs, and reduced their longevity. In a study to determine methods to inactivate viable *Syphacia muris* eggs, 100% inactivation occurred only with temperatures of 100°C for 30 minutes and ethylene oxide, although high killing rates with formaldehyde and chlorine dioxide suggested that these chemicals could be successful with adjustments to the protocol (Dix et al. 2004). Huerkamp and colleagues (2000) reported the eradication of S. muris without environmental decontamination, suggesting that the eggs in the environment may not have outlived the treatment period (fenbendazole in feed every other week for five treatments). S. obvelata eggs appear to be unstable, they are reported to survive only 42 hours under ideal conditions, and may be inactivated by drying or immersion in liquids (Chan 1952; Grice and Prociv, 1993). As noted above, *Syphacia muris* eggs are resistant to the most common disinfectants (Dix et al. 2004), and it is assumed that *Aspiculuris tetraptera* eggs have similar properties. Physical methods (e.g., scrubbing with detergent, steam cleaning, or painting) are thus most likely to be effective for environmental decontamination. Biosafety cabinets used to protect mice from aerosolized pathogens may actually be a route to widespread egg dissemination given that eggs shed in the cabinet are resistant to the routine disinfectants used to prevent transmission of other pathogens between cages. Tsuji and associates suggest the possibility of developing a mucosal vaccine for human and pig Ascariasis prevention. One of the current golds in the field of human and veterinary vaccines is the development of a noninvasive and practical route for administration via the mucosal surfaces (Tsuji et al. 2003). The same author previously developed a nasal immunization technique

with rAs14 that involvers protective immune responses against *Ascaris suum* infection (Tsiji et al. 2001).

Clifford and Watson (2008) revealed that, Long-recognized agents that remain in research facilities in the 21st century include parvoviruses of rats and mice, mouse rotavirus, Theiler's murine encephalomyelitis virus (TMEV), mouse hepatitis virus (MHV), and pinworms. The reasons for their persistence vary with the agent. The resilience of parvoviruses, for example, is due to their resistance to inactivation, their prolonged shedding, and difficulties with detection, especially in C57BL/6 mice. Rotavirus also has marked environmental resistance, but periodic reintroduction into facilities, possibly on bags of feed, bedding, or other supplies or equipment, also seems likely. TMEV is characterized by resistance to inactivation, periodic reintroduction, and relatively long shedding periods. Although MHV remains active in the environment for at most a few days, currently prevalent strains are shed in massive quantities and likely transmitted by fomites. (Clifford and Watson 2008). In the present study after treatment with Fenbendazole, Albendazole, Parazivet, and deworming drugs, all the rats were found parasite eggs negative, and a 100 % reduction was observed in all groups of rats. All the deworming drugs were found very effective to control both pinworm and ascariasis infections. Monthly treatment is needed to control or eliminate pinworm and Ascariasis reinfection in laboratory animals.

CONCLUSION

Pinworms and ascariasis remain prevalent in laboratory mice and rats, swine, and pigs. Rats are usually infected with pinworm *Syphacia muris* and mice with *Syphacia obvelata* and *Aspiculuris tetraptera* as well as *Ascaris suum* in both rats and mice in laboratory. Therefore, it was needed to eliminate worm infection in laboratory rats. For this purpose, thirty males and thirty females of Wistar rats (*Rattus norvegicus*) from DMR were randomly selected and 10 each were housed in cages separately for three deworming drugs fenbendazole, albendazole, and parazivet) were applied to selected groups of rats. Results found that 55 (91.67%) out of 60 were positive for Pin worm and Ascariasis eggs. Of these the highest density of mixed infection (Pinworm + Ascariasis) was found in 40 (72.73 %) followed by 8 (14.55 %) was pin worms positive and the lowest positivity was observed in 7 (12.73 %) of Ascariasis eggs. A total of 30 males and 30 females were tested, 28 (93.33 %) samples of males and 27 (90 %) samples of females were found parasite positive. After treatment with deworming drugs, all the rats were free from parasite eggs and a 100 % reduction was observed in all groups of rats and very effective to control both pinworm and ascariasis infection. And need to prevent from pinworms and Ascariasis reinfection in laboratory animals by monthly treatment of deworming drugs.

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