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Phytotoxicity Assessment of Different Animal Manure on Selected Vegetable Crops in Kashere Community, Gombe State, Nigeria

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ABSTRACT: The present study evaluated the phytotoxicity effect of poultry, goat and cow manure on Okra, Tomato and Pepper vegetables using appropriate standard methods. In the current study, of the three different manure collected and used on the three vegetable crops, GI value of <50% was recorded on Okra and Pepper seeds mounted on PM medium (48.51% and 41.36% respectively), GI value of between 50% and 80% was recorded on Okra, Tomato and Pepper seeds mounted on GM medium (64.76%, 79.77% and 73.70% respectively) also Pepper seeds mounted on CM medium (76.48%) and Tomato seeds mounted on PM medium (75.41%) while GI Value of >80% was observed on Okra and Tomato Seeds mounted on a CM medium (81.63% and 94.10% respectively). Overall result of all the animal manure evaluated, poultry manures was phytotoxic on okra and pepper seed and moderately phytotoxic on Tomato seed based on the plant bioassay evaluated. It is possible that other chemicals that were not analyzed in this study could potentially be responsible for the phytotoxic effects of poultry manure on the vegetable seeds, indicating that some curing in the form of composting or vermicomposting may be necessary to degrade or reduce the presence of phytotoxic compounds in the poultry manure used for the study before use as soil amendments and thus would help to render the resultant end product environmentally safer.

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Animal manure is animal excreta (urine and faeces) and bedding materials, which is usually applied to soils as a fertilizer for agricultural production (He, 2012). The beginning of the 20th century was characterized by the intensive confinement of animals, be it they cattle, pigs, birds, among others, aiming to increase productivity in small spaces. In this system, the animals remain at high stocking densities under full confinement until their slaughter (Koneswaran and Nierenberg, 2008; Steier and Patel, 2017). The use of organic fertilizer emphasizes maximum reliance on renewable local or farm resource. The advantage this fertilizer was cheap, improving soil arrangement, texture and airing, increasing the soils water preservation abilities and stimulating healthy root development (Twarog, 2006). Animal manure, such as chicken manure and cow dung, has been used for centuries as a fertilizer for farming. It can improve the soil structure (aggregation) so that the soil holds more nutrients and water, and therefore becomes more fertile. Animal manure also encourages soil microbial activity which promotes the soil's trace mineral supply, improving plant nutrition. It also contains some nitrogen and other nutrients that assist the growth of plants (Das *et al*., 2017). Similarly compost and/or biofertilizers have beneficial effects on plant growth and dry matter yield due to hormonal stimulation of root development and by supplying

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combined nitrogen (Gharib *et al*., 2008). As a consequence of this system, there is a high production of animal waste in the liquid or solid form (Bertora *et al*., 2008; Koneswaran and Nierenberg, 2008). Optimum utilization of animal manure requires knowledge of its composition in relation to its environmental implications and not only its positive benefits. Animal manure products like poultry manure have been observed to contain potentially harmful trace elements like arsenic, copper and zinc, which originate from the chemicals used to treat diseases in commercial chickens (Bolan *et al*., 2010). In fact, application on soil of no stabilized organic materials could affect both crops and the environment because of the presence of phytotoxic compounds (Butler *et al*., 2001). Organic vegetable production attempts to pursue multiple goals concerning influences on the environment, production resources, and human health (Islam *et al*., 2017; Ovsthus *et al*., 2015). Nutrition exerts influence on growth, yield, quality, and economics of vegetable crops. Imbalanced use and increasing cost of synthetic fertilizer are deterrents affecting productivity of these crops. Integrated nutrient management is a balanced use of inorganic fertilizers, organic manures, and crops in combination to maintain the desired crop production and maintenance of soil health (Hazra, 2007).

Phytotoxicity is one of the most important criteria for evaluating the suitability of compost for agricultural purposes and to avoid environmental risks before these composts can be recycled back to agricultural land. (Cooperband *et al*., 2003). Several researchers have used this phytotoxicity technique for different sources of waste products such as animal (eg. goat, cattle, pig) manure, compost and vermicompost products, and different sources of waste water (Ravindran and Mnkeni, 2017). High concentrations of salt and the release of organic acids into the composts are also correlated to inhibition of germination and growth. Phytotoxicity is often best evaluated by conducting germination or growth tests (Gariglio *et al*., 2002), but the test plants have to be chosen with care (Emino and Warman, 2004). Therefore, the objective of this study is to evaluate the phytotoxicity assessment of poultry, goat and cow manures on okra, tomato and pepper vegetables.

MATERIALS AND METHODS

Study Area: The study was be carried out in the Biological Garden of Federal University of Kashere with a GPS coordinates of $9^{0}52'40''$N, $11^{0}0'37''$E for the potting experiment, Phytotoxicity analysis was carried out in the Post Graduate Bioscience Laboratory of the Federal University of Kashere while Chemical Analysis was carried out at the Chemistry Laboratory Federal University of Kashere, Gombe State (Kolawole *et al*., 2021).

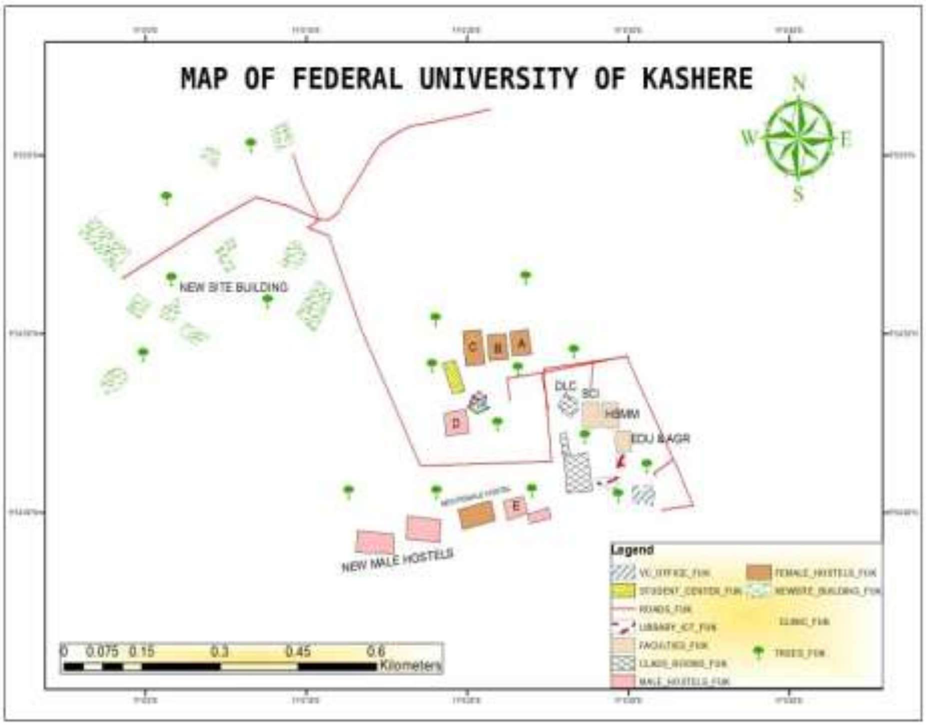

Fig 1: Location map of the study area

Materials: Tomato, Pepper and Okra seed, Polythene Pot, Soil, Animal manure (goat, cow and poultry), Petri dish, wahtman® No. 1 filter paper, weighing balance, distilled water, mechanical shaker, mortar and pestle, spatula, thread, meter-rule, incubator and writing materials.

Collection of Seeds: Vegetable Seeds (Okra, Tomato and Pepper) was gotten from the Plateau Agricultural Development Programme (PADP), Jos Plateau State.

Collection of animal manure: The animal manure comprising of cow manure, goat manure and poultry (chicken) manure was collected within Federal University of Kashere Community, Gombe State, Nigeria. After collection, the manure was then air dried at the Biology Garden of Federal University of Kashere, Gombe State. The dried animal manure was then kept under shade at room temperature for subsequent chemical and phytotoxic analysis.

Manure chemical content analyses: Before analysis, the dry animal manure was grounded to pass through a 2 mm sieve. The pH and electrical conductivity (EC) was analyse potentiometrically in deionized water at a ratio of 1:10 (w/v) as outlined by Mupambwa and Mnkeni (2015). Total nitrogen (TN) and total carbon (TC) was determined using the dry combustion method employing a Truspec C/N auto analyser (LECO, 2003). For the determination of total P, Na, Mg, Ca and heavy metals (Cr, Cu, Ni, Pb and Zn), the samples was then initially digested using aqua regia (3:1 v/v hydrochloric acid: nitric acid) in a MARS 5 microwave digester (CEM Corporation, Matthews, North Carolina). Following the digestion, the total P in the digests was determined using a continuous flow analyser (San 2 + + Skalar CFA, Skalar Analytical B.V. The Netherlands) whilst the rest of the elements was determine using the ICP-OES (Varian Inc., The Netherlands). The extractable P within the manure samples was then determined using the Olsen method which employs 0.5 M sodium hydrogen carbonate and was adjusted to a pH of 8.5 using sodium hydroxide (Schoenau and O'Halloran, 2006). The extracted P was then analysed automatically using a continuous flow analyser (San 2 + + Skalar CFA, Skalar Analytical B.V., The Netherlands) by employing the ammonium molybdate-antimony potassium tartrate-ascorbic acid method.

Phytotoxicity study: To study the phytotoxicity of the different animal manures, a seed germination bioassay was conducted using different vegetable crops. This assay was then determine using animal litter extracts prepared with distilled water (solid to water ratio of 1:10) (Ravindran and Mnkeni, 2016) and the seed germination bioassay was evaluated base on the method of Tiquia and Tam (1998). Distilled water was used as control. The water and manure mixtures was shaked on a mechanical shaker for 1 hour and then filtered using Wahtman® No. 1 filter paper. Thereafter, two pieces of Whatman® filter paper was placed inside a sterilized petri dish and wetted with 10ml of the animal (Poultry, cow and goat) manure extract on separate petri dish and 10 seeds of tomato (*Lycopersicon esculentum* L.), okra (*Abelmoschus esculentum* Mill.) and pepper (*Capsicum annum* L.) was then placed on top of the filter paper, each having three replicates and placed in an incubator with no light for five days. Seed germination, germination index (GI), relative seed germination (RSG) and relative root elongation (RRE) was then calculated from the measurements taken as shown in the equations 1, 2 and 3 respectively.

$$RSG\ (\%) = \frac{NSG_S}{NSG_C} * 100 \quad (1)$$

Where NSG_S = Number of seeds germinated in the sample extract; NSG_C = Number of seeds germinated in the control

$$RRE\ (\%) = \frac{MRE_S}{MRE_C} * 100 \quad (2)$$

Where MRE_S = Mean root elongation in the sample extract; MRE_C = Mean root elongation in the control

$$GI\ (\%) = \%\,SG * \%\,RE * 100 \quad (3)$$

Where SG = % seed germination; RE = % root elongation

The above index has proven to be the most sensitive parameter, capable of detecting low levels of toxicity which affect root growth, as well as high toxicity levels which affect the germination (Tiquia and Tam, 1998: Balasubramani *et al*., 2017)

Table 1: Summary of Seed germination test condition

Test Condition	
Test type	Static
Pre-treatment	No
Temperature	25-35^{0}C
Light	No
Test vessels	9cm diameter petri dish, whatman No. 3 filter paper
Test volume	10ml/dish
No. of Seeds	10/dish
Control	Distilled water
Test duration	120 hours (5 days)

Statistical analysis: The data reported in the present study are the means of three replicates (n = 3) and statistically analysed for the calculation of standard errors (S.E.). The data were subjected to analysis of variance (ANOVA) and where significant differences was observed the means was separated using Duncan's multiple range test at $P \leq 0.05$. All statistical analysis was conducted using the IBM statistical package, version 20.0 (Property of International Business Machines Corp).

RESULT AND DISCUSSION

Physicochemical Characterization

Electrical Conductivity and pH of Soil and the different animal manure samples used: The result of animal manure pH and EC are presented in (Table 2). The highest pH was recorded in the cow manure sample as 8.03 which indicate it to be slightly basic (alkalinity) with the lowest recorded in poultry (chicken) manure as 6.84 while 6.98 was recorded for goat. pH is a good indicator of any soil amendment quality and a neutral pH is usually preferred as most nutrients within the manure will be bioavailable in that range. Another good measure of manure quality is its potential to introduce salinity or toxic salts into soil, measured as Electrical conductivity. In the present study, the EC varies, 0.57 dS/m was recorded for cow manure, 0.88 dS/m for poultry manure and 0.94 dS/m for goat manure sample (Table 2) thus making all the three organic manure used in the study suitable for direct soil application.

Macro and Micro Nutrients: Table 2 shows the percentage value of total carbon (%) and total nitrogen (%) between all the three animal (organic) manures used in this study. Total carbon was lowest in cow manure with 4.13% and highest in poultry (chicken) manure with 6.38% indicating a significant difference in all the samples (animal manures). Similarly, there was significant difference in the total nitrogen with the lowest recorded in cow manure with 0.21% and the highest in poultry and goat manure sample with 0.32% and 0.38% respectively.

Table 2: Selected Physio-Chemical characteristics of the different animal manures used for the Study

Parameter	Poultry	Cow	Goat
pH	6.84 ± 0.03^{a}	8.03 ± 0.10^{c}	6.98 ± 0.01^{b}
EC (dS/m)	0.88 ± 0.00^{b}	0.57 ± 0.00^{a}	0.94 ± 0.00^{c}
Total C%	6.38 ± 0.00^{c}	4.13 ± 0.01^{a}	6.31 ± 0.00^{b}
Total N%	0.32 ± 0.00^{b}	0.21 ± 0.00^{a}	0.38 ± 0.00^{c}

Values are mean ± S.E. Means in a row with different superscripts significantly different at p<0.05

The result of the net available phosphorus in the organic manure in this study was presented in table 3 and it shows a significant difference in the net P present in the samples (poultry, cow and goat manure) ranging from 45.23mg/kg - 90.28mg/kg. This study only looked at three cations: Na, Mg and Ca (Table 3). It was observed that all the cations were significantly different in all the samples used in the study. Cations are secondary nutrient that also influences chemical properties which also have impact on plant growth. All the organic manure and the soil used for this study had a very low concentration of cations. The heavy metal content (Zn, Cu, Cr, Ni and Pb) in the samples (PM, CM and GM) was investigated (Table 3). The heavy metals investigated in this study are significantly minimal which indicate minutes heavy metals present in all the studied samples compared to high range of values of heavy metals reported by some researchers.

Table 3: Selected cations and heavy metals concentrations in the different animal manure used for the Study

Parameter (mg/kg)	Poultry	Cow	Goat
P	45.23 ± 0.00^{a}	51.85 ± 0.00^{b}	90.28 ± 0.00^{c}
Na	78.63 ± 0.14^{b}	81.46 ± 0.14^{c}	54.13 ± 0.08^{a}
Ca	113.90 ± 0.15^{b}	115.36 ± 0.06^{c}	90.40 ± 0.11^{a}
Mg	13.05 ± 0.00^{b}	12.32 ± 0.00^{a}	18.64 ± 0.00^{c}
Zn	0.69 ± 0.00^{a}	0.77 ± 0.00^{b}	2.14 ± 0.00^{c}
Cu	0.10 ± 0.00^{b}	0.11 ± 0.00^{a}	0.31 ± 0.00^{c}
Cr	0.06 ± 0.00^{a}	0.07 ± 0.00^{b}	0.09 ± 0.00^{c}
Ni	0.005 ± 0.00^{a}	0.008 ± 0.00^{b}	0.016 ± 0.00^{c}
Pb	0.005 ± 0.00^{b}	0.002 ± 0.00^{a}	0.007 ± 0.00^{c}

Values are mean ± S.E. Means in a row with different superscripts significantly different at p<0.05

Phytotoxicity Evaluation: A number of toxicity bioassays have been developed to evaluate the phytotoxicity of animal manures, several researchers have used this phytotoxicity technique for different sources of waste products such as animal manure, compost and vermicompost products.

Relative Seed Germination (RSG): The results show that across the three vegetable crops and the three different animal manures used, the RSG % was within the range of 61.57% - 88.88% for Okra, 80.55% - 96.29% for tomato and 65.07% - 91.26% for pepper (Table 4). Among the three different manure used (Poultry, Cow and Goat), RSG (%) values of <80%

was recorded in Okra and Pepper seeds mounted in PM and Okra seeds mounted in GM medium while RSG >80% was recorded in Okra, Tomato and Pepper seeds mounted in CM medium, Tomato seeds mounted in PM and also Tomato and Pepper seeds mounted in GM medium (Table 4).

Table 4: Relative seed germination (%) of different Vegetable crops in extracts of different animal manures

Manure Type	Okra	Tomato	Pepper
Control	100 ± 0.00^{c}	100 ± 0.00^{a}	100 ± 0.00^{b}
PM	61.57 ± 3.24^{a}	80.55 ± 10.01^{a}	65.07 ± 4.20^{a}
CM	88.88 ± 6.41^{bc}	96.29 ± 3.70^{a}	84.92 ± 8.28^{ab}
GM	76.84 ± 6.48^{ab}	83.79 ± 4.41^{a}	91.26 ± 13.34^{ab}

Values are mean ± S.E. Means in a column with different superscripts significantly different at $p<0.05$
Key: PM= Poultry Manure, CM= Cow Manure, GM= Goat Manure

Relative Root Elongation (RRE): The result of RRE was reported in (Table 5). Like the RSG, no significant differences were observed between poultry manures on RRE % (Table 4). For Okra, the RRE ranged from 74.19% − 92.42%; whilst for Tomato and Pepper it ranged from 94.97% − 99.21% and 64.92% – 89.70% respectively. RRE > 80% was recorded in almost all the vegetable crops and in the an imal manure used except Pepper and Okra mounted in poultry manure medium having RRE <80% respectively.

Table 5: Relative root elongation (%) of different Vegetable crops in extracts of different animal manures

Manure Type	Okra	Tomato	Pepper
Control	100 ± 0.00^{c}	100 ± 0.00^{a}	100 ± 0.00^{b}
PM	74.19 ± 3.66^{a}	95.64 ± 9.76^{a}	64.92 ± 4.90^{a}
CM	92.42 ± 4.70^{bc}	99.21 ± 9.08^{a}	89.70 ± 9.24^{b}
GM	84.46 ± 4.37^{ab}	94.97 ± 3.37^{a}	81.73 ± 0.26^{ab}

Values are mean ± S.E. Means in a column with different superscripts significantly different at p<0.05
Key: PM= Poultry Manure, CM= Cow Manure, GM= Goat Manure

Germination Index (GI): The investigated GI is represented in (Table 6). Phytotoxicity quantification is mainly based on germination index (GI) and this value is derived from the performance of crop seedlings, relative seed germination and relative root elongation during the assay period. Some researchers suggested that GI values less than 50% indicated a phytotoxic medium while those between 50% and 80% indicated moderate phytotoxicity and GI values greater than 80% indicated lack of phytotoxicity. In the current study (Table 6), of the three different manure collected and used on the three vegetable crops, GI value of <50% was recorded on Okra and Pepper seeds mounted on poultry manure medium (45.81% and 41.83% respectively) indicating that PM extract is phytotoxic to Okra and Pepper Seeds, GI value of between 50% and 80% was recorded on Okra, Tomato and Pepper seeds mounted on GM extract, Pepper seeds mounted on CM extract and Tomato seeds mounted on PM extract indicating moderate phytotoxic medium while GI Value of >80% was observed on Okra and Tomato Seeds mounted in a CM medium indicating limited or no phytotoxicity.

Table 6: Germination index (%) of different Vegetable crops in extracts of different animal manures

Manure Type	Okra	Tomato	Pepper
Control	100 ± 0.00^{d}	100 ± 0.00^{a}	100 ± 0.00^{b}
PM	45.81 ± 4.03^{a}	78.23 ± 16.48^{a}	41.83 ± 0.51^{a}
CM	82.27 ± 3.60^{c}	95.50 ± 9.66^{a}	77.36 ± 13.88^{b}
GM	64.10 ± 4.76^{b}	79.49 ± 4.41^{a}	74.62 ± 11.00^{b}

Values are mean ± S.E. Means in a column with different superscripts significantly different at p<0.05
Key: PM= Poultry Manure, CM= Cow Manure, GM= Goat Manure

Physiochemical characterization: The result of the nutrients and heavy metal analysis in this study is termed as the physiochemical characterization, pH is a good indicator of any soil amendment quality and a neutral pH is usually preferred as most nutrients within the manure will be bioavailable in that range. Ontario Ministry of Agriculture, (2009) suggested that plant growth is optimal at a pH of between 5.8 and 6.5 and sometimes up to 7.5 depending on plant species, thus making the Poultry (chicken) and goat manure used in this study potentially are good soil amendments, while the cow manure used was slightly basic at 8.03 which can also be used as slightly basicity is advantageous than acidity. Another good measure of manure quality is its potential to introduce salinity or toxic salts into soil, measured as Electrical conductivity. High EC indicate high salt concentrations which can result in disruption of soil physiological processes in the plant (Cho *et al.*, 2017). In this study, the EC varies and thus making all the three organic manure used in the study suitable for direct soil application. If organic manure EC is above 6.0 dS/m, the amended soil should be leached with water before planting seeds and only few crops can tolerate such salt level (Ozores-Hempton *et al*., 2013). Physio-chemical parameter values were recorded at appreciable levels in all the animal manure extracts. All samples showed adequate concentration of total macro and micro nutrients. Irshad *et al*. (2013) and (Wang *et al*., 2013), suggested the investigation of metal content in animal manures because this can provide useful information to predict their bio-availability and also potential for contamination of soils. In this study, the presence of heavy metals Zn, Cr, Cu, Ni and Pb in the animal manures and soil sample was investigated (Table 2). The heavy metal concentrations (mg/kg) detected in most of the collected animal manures were very minute ranging from Zn to be highest in goat manure with 2.14 mg/kg while the lowest was recorded in poultry manure to be 0.69 mg/kg, Cu was highest in Goat manure with 0.31mg/kg and the lowest was recorded in poultry manure with 0.10 mg/kg, Cr was highest in goat manure sample with 0.09 mg/kg and lowest in poultry manure with 0.06 mg/kg, Ni was highest in goat manure with 0.016 mg/kg and lowest in poultry manure sample with 0.005 mg/kg and Pb was highest in goat manure with 0.007 mg/kg and lowest in cow manure with 0.002 mg/kg unlike what was reported by Balasubramani *et al*., (2017) on chicken manure collected from different farm houses which indicate the highest value of Zn in one of the chicken manure sample to be 845.1mg/kg, Cu to be 134.4mg/kg, Cr to be 33.8mg/kg, Ni to be 25.7mg/kg and Pb to be 107.1mg/kg which is still within acceptable level. The U.S. Environmental Protection Agency (USEPA) does not restrict bio-solid applications based on metal content (mg/kg) until the material has a total individual metal concentration of Zn > 2800; Cr > 1200; Cu > 1500; Pb > 300 and Ni > 420 (USEPA, 1994). The recorded heavy metal content in the animal manures used for these studies were thus within acceptable levels according to the USEPA range. The exchangeable bases observed in this study can be important in improving the fertility of soils when applied to the soil at optimized rates (Dikinya and Mufwanzala, 2010).

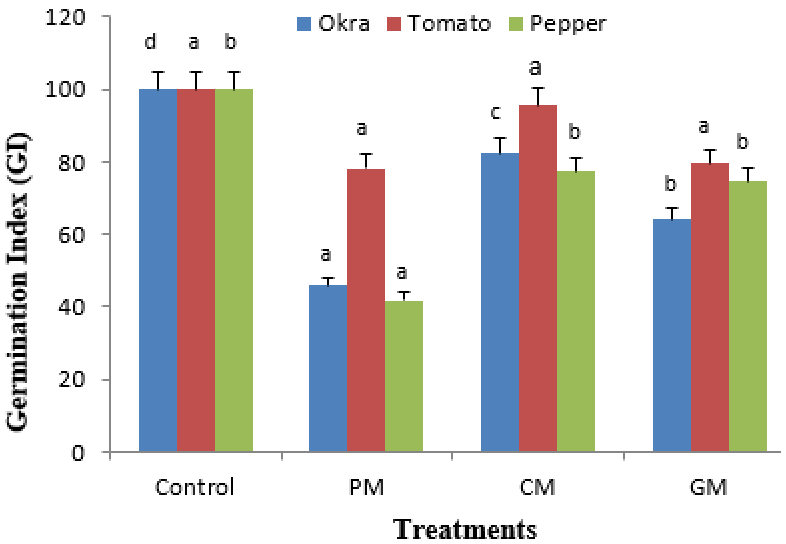

Fig 2: Chat showing the effect of different animal manure extracts on the Germination index (%) of different Vegetable crops

Phytotoxicity Evaluation: Result of Phytotoxicity quantification is mainly based on germination index (GI) and this value is derived from the performance of crop seedlings, relative seed germination and relative root elongation during the assay period. Paradelo *et al*. (2008) suggested that GI values less than 50% indicated a phytotoxic medium while those between 50% and 80% indicated moderate phytotoxicity and GI values greater than 80% indicated lack of phytotoxicity. Studies by Majlessi *et al*. (2012) and Ravindran *et al*. (2016) also supported this categorization. Evaluation of phytotoxicity can also be used to determine the inhibition of growth due to release of phytotoxins by one plant that influence another plant growth, as in the study of kolawole *et al*., (2023) where it was observed that the Germination index (GI) of all three-test species were highly suppressed by aqueous leaf extract of *Senna singueana* with increasing concentrations thus, indicating high phytotoxic content present in *Senna singueana*. Phytotoxicity in manure has been attributed to the presence of heavy metals, ammonia, salts and low molecular weight organic acids that might not have been metabolized (Zucconi *et al*., 1985). In the current study (Table 5), of the three different manure collected and used on the three vegetable crops, GI value of <50% was recorded on Okra and Pepper seeds mounted on poultry manure medium (45.81% and 41.83% respectively) indicating that PM extract is phytotoxic to Okra and Pepper Seeds, GI value of between 50% and 80% was recorded on Okra, Tomato and Pepper seeds mounted on goat manure (GM) extract also Pepper seeds mounted on cow manure (CM) extract and Tomato seeds mounted on PM extract indicating moderate phytotoxic medium while GI Value of >80% was observed on Okra and Tomato Seeds mounted in a CM medium indicating limited or no phytotoxicity. It was interesting to note that, as the EC and total carbon of the animal manure increases, the RRE and GI also decreases for all the vegetable crops as observed for goat and poultry manure in this study. However this was unlikely the case when these manures were applied to the soil which also go in line with the study of Mufwanzala and Dikinya (2010). Among all the animal manure, poultry manures were either phytotoxic (< 50% GI) or moderately phytotoxic (< 80% GI) on all the vegetable crops used in this study. This result is in agreement with the findings of Balasubramani *et al.*, (2017) on chicken manure collected from different farm houses which shows some of the poultry manure samples recording a germination index of less than 50% for the different crops evaluated. It is also possible that other chemicals that were not analyzed in this study could potentially be responsible for the phytotoxic effects of poultry manure on the vegetable seeds, indicating that some curing in the form of composting or vermicomposting may be necessary to degrade or reduce the toxic effects of phytotoxic compounds before the manures can be safely used as soil amendments. This could be done by the combined composting and vermicomposting of poultry manure mixed with other organic bulking materials such as soil which can metabolize these phytotoxic compounds whilst forming organ-metallic complexes with heavy metals, reducing their bioavailability and thus rendering the resultant end product environmentally safer.

Conclusion: The results of this study indicated that the animal manures, however differed significantly in their physio-chemical and phytotoxic properties. The phytotoxicity observed on most especially poultry manure on okra and pepper vegetables pointed to the need for further biodegradation through composting to improve nutrient content and reduce the phytotoxicity to levels that can be tolerated by the plants. There is also need to evaluate the actual impacts of poultry manure on crop phytotoxicity under field conditions when the manures are applied directly to the soil.

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