Optimization of Anti-microbial and Pesticidal Efficacies of bio-slurry using *Terminalia b*. and *Acanthaceae spp*. extracts

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***Abstract:*-Over time, many plant pests have grown resistant to commercial pesticides. More pesticides are thus used to combat their effects leading to agricultural expenses and environmental pollution. Biogas slurry is known to have a natural pesticidal effect. This study aimed at analyzing the effects of using two indigenous biogas additives (*Terminalia b.* and *Acanthaceae spp*.) in hastening the antimicrobial and pesticidal activity of bio-slurry. Kitchen waste was used as the biogas substrate for a retention period of 30 days. The extracts were characterized for pesticidal components before subjecting to antimicrobial analysis. Both gram positive (*Staphylococcus aureus*) and gram negative (*E. coli*) bacteria along with *Candida albicans* fungus were used for antibacterial and antifungal tests respectively. In vitro efficacy tests were carried out on aphids and fall armyworms and the toxicity studies (acute dermal toxicity and acute dermal irritation) carried out on lab animals. The results indicated abundance of organophosphates with traces of chlorides, organochlorides and carbamates in the test samples. There was significant antifungal and antibacterial properties of the additive samples compared to the control sample ($p \leq 0.05$, n= 8). The samples were effective in the control of aphids (*Brassica alboglabra*) as observed in the in vitro analysis. Suppression of fall armyworm (*Spodoptera frugiperda*) was not satisfactory enough. The samples were dermally non-toxic, neither do they induce dermal irritation.**

Key words: **pesticides, anti-microbes, bio-slurry, *Terminalia b., Acanthaceae spp.***

I. INTRODUCTION

Use of pesticides is a crucial requirement for optimal agricultural yields. Pesticides play a key role in mitigating the effects of insects, fungi, bacteria, rodents, termites and any other undesired organism that affect normal growth and production of crops (Staley *et al*., 2015). Different crops depend on pesticides differently depending on many factors such as the immediate soil, environment, season and climate (Guo *et al.,* 2015). Additionally, different plants require different pesticides at varying periods and concentrations (Kwon *et al.,* 2018).

Most of the popular pesticides used are synthetic. Whether organic or inorganic, these pesticides have affiliated side-effects to non-target organisms including the farmer. The most notable effect is increased air and water pollution (Vaseem and Banerjee, 2016; Osano *et al*., 2009).While the inorganic pesticides are heavily laden with toxic heavy metals such as lead, arsenic and mercury (Magu *et al*., 2016; Maghanga *et al*., 2013), the organic ones also have appreciable concentrations of halo-alkanes (Magu *et al.,* 2016), which are equally lethal (Magu *et al.,* 2016). Incorporation of these heavy metals in the food chain is a source of carcinogens to human beings (Osano *et al*., 2004). On the other hand, halo-alkanes degrade upon irradiation by sunlight to produce reactive radicals (Chaka and Osano, 2019). Methyl radical is one such radical from degradation of halo-alkanes and is responsible for initiation of ozone depletion process (Claxton *et al*., 2019). Other halide radicals are responsible for global warming (Erickson *et al.,* 2014).

There is a growing concern over the efficacy of current insecticides. A lot of pesticide is applied to mitigate the effects of few pests which is uneconomical. Exposure to large concentrations of pesticides is also known to have various toxicological effects to the person handling the pesticide (Bevan *et al*., 2017). Different types of pesticides with varying mode of action against insects, bacteria, rodents, fungi, herbs and other organisms can equally act on human skin or body openings (Bevan *et al*., 2017). Most natural pesticides such as nicotine and neem extracts are usually less toxic to humans (Nile *et al.,* 2019). It is thus feasible to plough into mass production of natural pesticides.

Biogas slurry is an excellent organic fertilizer with the potential to keep away pests from plants. The plant nutrients from a biogas digester are in a more soluble form for easy uptake by roots (Meyer *et al*., 2018). Organic manure is composed of a myriad or compounds which potentially complexate plant nutrients. In this form, the nutrients are immobilized and cannot be easily up taken by plants. Degradation of organic matter in biogas digester by anaerobic bacteria frees nutrients from these complex organic matrices (Singla *et al*., 2015). Nutrients in ionic form can easily be up taken by plants.

Biogas slurry has previously been applied and successfully kept away fall armyworms from maize and striga from sorghum (Mekonnen, 2017).Bio-slurry has also been used to control tobacco mosaic disease, a viral disease (Nicholas and Xu, 2013). Bio-slurry is rich in organic compounds and phosphates and carbonates (Jacob and Banerjee, 2017). Formation of organophosphates and carbamates is thus likely depending on the environment and composition of the biogas slurry. Both organophosphates and carbamates are lethal pesticides whose mode of action involve preventing nerve cells of pests from communicating with each other (Eaton *et al*., 2008). These pesticides inactivate the acetyl cholinesterase (AChE) enzyme found in neural transmitters of pests eventually leading to wear out of the pest and death (Eaton *et al*., 2008).

Terminalia b. leave and *Acanthaceae spp*. bark extracts were anciently used by the Aandia and Maasai communities of Kenya respectively to hasten saccharification of biomass for production of ethanol and fast fermentation of porridge. These bio-catalysts can potentially quicken the availability of free nutrients from organic manure. In the process, organophosphate and carbamate pesticidal compounds can be quickly developed. These are natural pesticides which are associated with less toxicity and are cheap to prepare.

II. MATERIALS AND METHODS

2.1 Design of Experiment

An independent measures design was carried out using bio-slurry samples. Three bio-slurry samples obtained from a control substrate of kitchen waste and substrate containing *Acanthaceae spp.* and *Terminalia b.* extracts dosed at 5%$^{w}/_{v}$ were characterized before testing for their pesticidal activity. The samples were characterized for physical-chemical parameters, functional groups, phosphates, chlorides and carbamates by both wet chemistry and spectroscopy. Change in dissolved oxygen and presence of pesticidal compounds was also monitored before subjecting the samples for antimicrobial analysis using both gram negative and positive bacteria as well as fungi. Efficacy tests were conducted using aphids and fall armyworms while acute dermal toxicity and acute dermal irritation tests conducted on rats and rabbits respectively.

Characterization and efficacy tests were carried out in Maasai mara university, Kenya while UV VIS analysis for phosphates was done at Vaal university of technology, South Africa.

2.2 Materials

2.2.1 Chemicals

All chemicals used were lab grade except for analytical grade reagents which are hereby specified. All chemicals were sourced from Sigma-Aldrich.

Sodium hydroxide pellets, lead nitrate, silver nitrate, sodium sulphide, n-hexane, acetonitrile, isopropyl alcohol, absolute ethanol, furfuric acid, methyl red indicator, hydrochloric acid. Potassium sulfate, copper sulfate pentahydrate, sulfuric acid, alundum boiling chips, nitric acid, quinoline, sodium molybdate, magnesium sulfate heptahydrate and ammonia solution.

The analytical reagents used include; potassium bromide, ammonium metavanadate, ascorbic acid and hydrazine sulphate.

2.2.2 Antimicrobial tests

Muller-Hinton agar (Sigma-Aldrich), nutrient agar, potato dextrose agar, sterile distilled water, ethanol.

2.2.3 Equipment

Fourier transform infra-red (Shimadzu), pH meter (Hanna G-114), Ultraviolet visible spectrometer (Jenway-6850), Autoclave (Wisconsin Aluminum Foundry, UL 6P38, 25X-2), Incubator (Omega I-52, PNP 9052).

2.3 Methods

2.3.1 Characterization of pesticidal extracts

The pesticidal extracts were characterized for physical chemical parameters, functional groups as well as pesticide inhibiting compounds. Quantitative analysis was done for phosphorus (as total phosphorus, total phosphoric acid and free phosphates) while chlorides, organophosphates, organochlorides and organo-carbamates were screened for presence using wet chemistry methods. These tests are outlined below;

pH and Electrical Conductivity

pH and electrical conductivity were conducted using a pH meter and conductivity meter respectively.

Chemical Oxygen Demand (COD) and Bio-chemical Oxygen Demand (BOD)

COD was conducted using an oxygen meter and the value of dissolved oxygen analyzed again after five days as BOD. During this period, the bottles containing the samples were well stoppered and covered in a dark paper then stored at 20^0C.

Total Solids (TS) and Volatile Solids (VS)

10.000 g of sample was weighed, M_1 using an Analytical balance and then placed in an oven conditioned at 105^0C for 6 hours before removing, cooling (in a desiccator) and reweighing. The new mass was recorded as M_2.

$$\%TS = \frac{M2}{M1} \times 100\% \qquad \textbf{(1)}$$

The procedure was repeated using a similar mass, M_1 of sample but heating done for 1 hour at 540^0C. The new mass was recorded as

$$\%VS = \frac{M3}{M1} \times 100\% \qquad \textbf{(2)}$$

IR Functional Group analysis

The extracts will then be heated slowly at 60^0C until all the water was dried. The samples were then cast into pellets using potassium bromide pellet before analyzing for functional groups using IR Spectrometer.

Test for chloride groups

This method is by Hansen and Thunemann, 2016.

20%$^{v}/_{v}$ sample solution in distilled water was distilled to get at least a third of the initial solution. The clear distillate was cooled before adding 1M $Pb(NO_3)_2$ solution dropwise till in excess. On a separate test-tube, the reaction was confirmed by adding 1M $AgNO_3$ dropwise till in excess. Precipitation was monitored and recorded.

Qualitative test for organophosphates, organochlorides and carbamates

20ml of bio-slurry sample was added onto 100ml n-hexane in a round bottomed flask and swirled to homogenize. 5.0g of sodium sulphide was then added before refluxing at about 80^0C for 1 hour. The solution was then filtered and the filtrate further separated using a separating funnel before extracting twice with 50ml and 25ml acetonitrile solution. The layer containing acetonitrile was further mixed with 500ml distilled water and 3ml saturated sodium sulphide solution before shaking thoroughly in a separating funnel with n-hexane. The supernatant was evaporated off using a water bath at 50^0C. The remaining mixture was concentrated to about 5ml before testing for organochloro, carbamate and organophosphates by specific color test.

a) Organochloro test: The residue was treated with Isopropyl alcohol and observed.
b) Organophosphate test: The residue of 1ml in 5ml ethanol was treated with Potassium hydroxide and the color reaction was observed.
c) Carbamate test: Residue of 1ml in 5 ml ethanol was treated with 1 drop Furfural and 1 drop of HCL, shaken for one minute and the color reaction was observed

Total Phosphorus analysis

The method is according to Kimie *et al*., 2013 and Masayoshi, 1988.

A filter paper was weighed and stored in a desiccator. 3.000g of sample will be dissolved in 40.0 ml of distilled water and a different filter paper used to filter the mixture. 45 ml of 10% $MgSO_4.7H_2O$ was added to the filtrate followed by 150 ml of 2M NH_3 slowly while stirring. A white precipitate was formed and the mixture allowed to stand at room temperature for 15 minutes. The precipitate was then quantitatively transferred to the pre-weighed filter paper and washed with two 5 ml portions of distilled water and two 10 ml portions of 95% ethanol. The precipitate was then spread on a watch glass for 8 hours and dried in the oven at 100^0C for 1 hour. The precipitate was again cooled for 15 minutes before reweighing.

Total Phosphoric Acid (TPA) by Quinoline gravimetric analysis

The method is according to Kimie *et al*., 2013 and Masayoshi, 1988.

1.0g of sample was weighed into a round bottomed flask. Catalysts 10.0g potassium sulphate and 1.0g of hydrated copper sulfate was added followed by 20.0ml of concentrated sulphuric acid together with alundum boiling chips. The mixture was allowed to digest until white fumes clear the flask. Thereafter, 100ml of distilled water was added until the color of the mixture starts to change. The total volume was recorded as V_1. 10.0ml of this solution (V_2) was mixed with 10.0ml of conc sulphuric acid/nitric acid mixture (1:1) before adding into 50.0ml of Quimosiac solution (prepared using quinoline and sodium molybdate). The mixture was then filtered onto a pre-weighed filter paper. The filter paper was then dried at 220^0C for 30 minutes and reweighed again.

$$(T\text{-}P_2O_5)\ \% = A \times 32.07 \times 100/1000 \times V_1/V_2 \times 1/W \quad \textbf{(3)}$$

Where A is mass of precipitate and W is weight of sample in grams.

Test for free phosphates

UV VIS phosphates standards were prepared by dissolving 1.7081 g of ammonium molybdate/ Ammonium metavanadate and ascorbic acid (5.82g/300ml distilled water) in 150 ml warm water. The solution was cooled before diluting to 250 ml. 0.125 g of hydrazine sulphate in 100 ml distilled water was added. Analyte samples were diluted by a factor of 10 and added the conditioning reagent before measuring the absorbances at 830-860 nm against those of the blank and standards.

2.3.2 Antimicrobial analysis

Antimicrobial studies were conducted for both Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli). C. albicans* strain was used for antifungal analysis. All aseptic techniques were considered to minimize the contamination rates.

Media preparation

28.0g of Muller-Hinton's agar media was dissolved into 600ml of sterile distilled water in a media dispensing bottle. The mixture was gradually boiled to completely dissolve the media. Caution was taken not to break the media bottle by loosening the bottle stopper occasionally to avoid pressure build up. The media was then sterilized by autoclaving along with petri-dishes and all apparatus to be used at 121^0C and 15 psi pressure for 15 minutes. The media was allowed to cool to 45^0C before dispensing in sterile petri dishes. The media plates were allowed to cool, inverted and stored in the refrigerator at 4^0C for 24 hours.

Antimicrobial sensitivity tests

Sterile media plates were sub-divided into six equal parts using a marker pen and labelled accordingly. The test microbes were then spread aseptically on different media plates to prevent cross-contamination. Sterile octodiscs impregnated with different bio-slurry samples were then placed on the surface of the plates. The plates were then inverted and incubated at 37^0C for 24 hours. The inhibition zones were noted and recorded in millimeters.

2.3.3 Efficacy analysis

Fall armyworms and aphids were collected from around the university together with their immediate ecological niche (maize leaves and kale leaves respectively). The fall armyworms were classified in groups of ten according to their sizes. Three strata for each of the bio-slurry sample were obtained. The insects were then put into open plastic beakers together with the leaves and 5ml of the bio-slurry sprayed onto the beakers. A fourth strata involving the insects and their niche but without the additives was also included as a control experiment. Onto this strata, 5ml distilled water was sprayed.

For the aphids, three strata containing 50 aphids were obtained plus a control. The aphids were also kept in open beaker and sprayed with the pesticidal samples and distilled water for the control experiment. The behavior of the insects was observed for the initial 30 minutes and their population monitored after every 3 hours for the next 48 hours.

2.3.4 Toxicological analysis

Acute dermal toxicity

Six white healthy rats were obtained from the university farm and labelled on their ears by marking with different inks. The rats were separated in a probation cage and observed for any abnormalities for 24 hours. The rats were then shaved off their fur for around 5cm by 3cm at their back to expose the skin and left for 24 hours. 2ml of the bio-slurry samples were then applied onto the shaved part gently and a non-irritating gauze patch mounted using zinc oxide adhesive. The color of the skin together with behavior of the rats were observed for the next 24 hours after which the bandage was removed. The population of the rats was also assessed.

Acute irritational toxicity

The above procedures were repeated using white rabbits on a $6cm^2$ patch of skin using 0.5ml of the residual sample. The behavior of the rabbits was observed keenly and the change in skin color after 3 hours also monitored. The chart below (**table 1**) was used to classify the level of acute irritational toxicity.

Table 1: The levels of scaling dermal irritation effect

Dermal irritation effect		Score
Oedema formation	**Erythema and Eschar formation**	
No oedema	No erythema	0
Very slight oedema (barely perceptible)	Very slight erythema (barely perceptible)	1
Slight oedema (edge of area well defined by definite raising)	Well defined erythema	2
Moderate oedema (raised by approximately 1mm)	Moderate to severe erythema	3
Severe oedema (raised by more than 1 mm and exceeding beyond area of exposure)	Severe erythema (beef redness) to eschar formation preventing formation of erythema	4

Dermal irritation scales chart

2.4 Statistical Analysis

Several statistical tools including the mean, median, standard deviation and correlation were analyzed using Ms Excel and Originlab statistical packages.

III. RESULTS AND DISCUSSIONS

3.1 Characterization of Pesticide Samples

3.1.1 Physical-chemical and bio-chemical parameters

The appearance of pesticides is crucial in determining many key decisions such as its primary mode of application, storage and conveyance, durability and possible carrier solvents (formulations) to use in different situations (Anstrom *et al.,* 2012).The characteristics of pesticides go a long way in determining their efficacy to the target organisms. The environmental pollution effects of a pesticide are also related to their characteristics (Qu *et al.,* 2016). **Table 2** below summarizes the average values of properties of the pesticidal samples analyzed.

Table 2: Physical-chemical and bio-chemical properties of pesticidal samples

Parameter	Samples		
	Control	*Terminalia b.*	*Acanthaceae spp.*
pH	5.880 ± 0.010	5.860 ± 0.003	5.910 ± 0.010
E. Conductivity (mS)	4.520±0.003	4.770±0.021	5.250±0.027
Total Solids (g/L)	4.653±0.616	3.664±0.217	6.435±0.899
Volatile Solids (g/L)	4.312±0.669	3.552±0.813	4.892±0.677
COD (%)	7.800±0.013	7.400±0.100	7.520±0.100
BOD (%)	6.774±0.123	6.889±0.214	6.901±1.010

Physical-chemical and bio-chemical properties of the pesticidal samples

All samples were found to be in their optimal pH range of between 5-7 (Zhuang *et al*., 2016). Increase in pH values above 7.0 renders the pesticide basic triggering alkaline hydrolysis process (Masbou *et al.,* 2018). Alkaline hydrolysis is a common reaction that occur whereby insecticides and miticides at pH values above 7.0 dissociate or fragment into other ions which lack any pesticidal activity (Masbou *et al.,* 2018). The process increases with increase in alkalinity (Masbou *et al.,* 2018). *Acanthaceae spp.* samples had the highest conductivity values ($5.250 \pm 0.027 mS$) indicating possibility of more chloride residues. High conductivity values imply the pesticidal residues are more soluble in water thus have higher chances of moving further away with the water. This effect is suitable as it increases its exposure to pests. The sample total solids were quite low with *Acanthaceae spp*. samples having the highest content ($6.435 \pm 0.899 g/L$) while *Terminalia b*. samples had the lowest ($3.664 \pm 0.217 g/L$).More solid content reduce the transportation of the pesticide formulation and by extension minimize water pollution. The volatile solid contents of the pesticidal samples constituted a large composition of their total solid indicating the pesticides were volatile in nature. Both the control sample and *Terminalia b*. samples had more effective volatility (about 93% and 94% respectively). These samples would require spraying at cold temperatures or during dawn and dusk when the sun is not quite hot to minimize volatilization into the atmosphere. The variations in COD and BOD values were quite minimal as expected in most pesticides. Large variations in COD and BOD values implies presence of more aerobic microorganisms (Gonzalez *et al.,* 2015). These organisms convert oxygen to other compounds depleting dissolved oxygen. Most pesticides, especially organochlorines, kill bacteria and other micro-organisms therefore inhibiting oxygen depletion rates (Ziegler *et al*., 2019).

3.1.2 Functional groups analysis

All samples were found to have similar peaks indicating very high chances of resemblance. The collated FT-IR spectra of the three samples is illustrated by **figure 1** below.

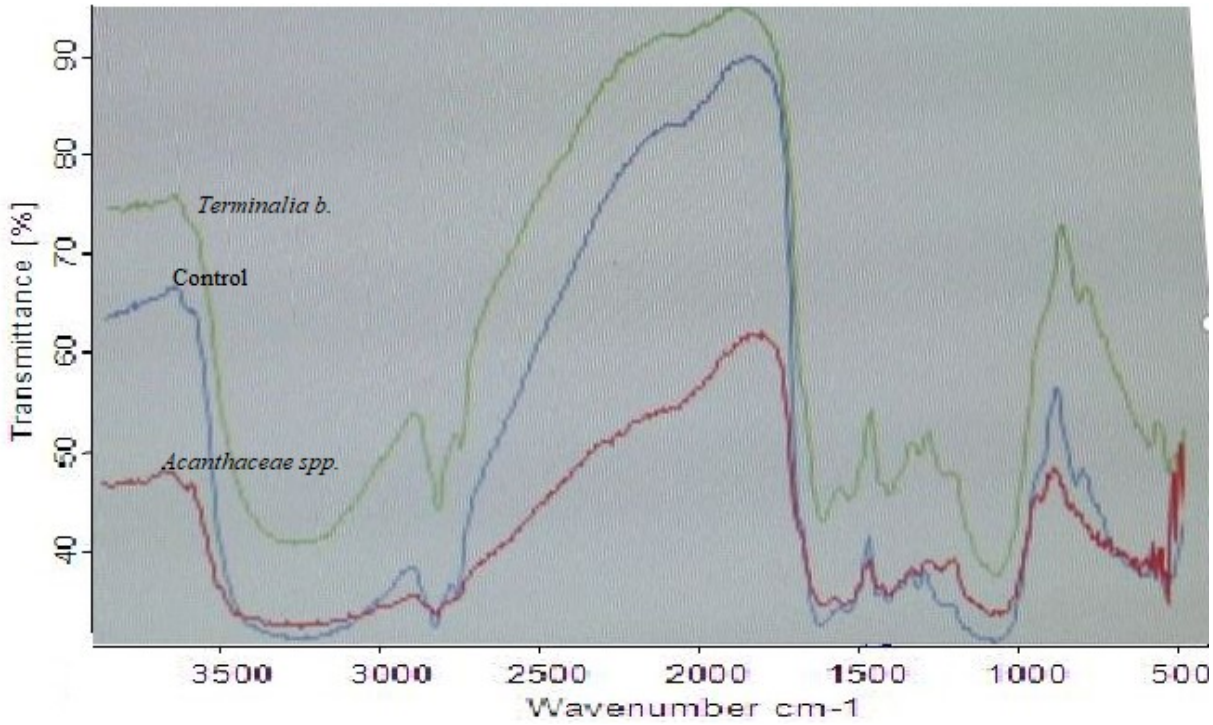

Figure 1; FT-IR spectra of pesticidal residues

All the three samples had wide peaks between 2800-3400cm^{-1} ($O\text{-}H_{RCOOH}$) and at 1680cm^{-1} ($C{=}O_{stretch}$) indicating presence of weak carboxylic acids. sp^3 $C\text{-}H_{stretch}$ peaks were observed at about 2850cm^{-1} while there were mild $C\text{-}OH_{stretch}$ peaks at about 1033cm^{-1}. Presence of organo-carbamate pesticidal residues was supported by symmetric N-H peaks at 1380cm^{-1}, $C\text{-}O\text{-}C_{vibration}$ peak at about 970cm^{-1} and the carbamate (CNH) peak between 1616 and 1646cm^{-1}. There were sharp P-C peaks at 1350cm^{-1} and mild PO_4^{3-} peaks at 870cm^{-1} to indicate presence of organophosphate residues. Presence of organochlorides was supported by the sharp peaks at 560cm^{-1}. All samples appeared to be quite conjugated owing to the intense peaks at the fingerprint region (Alfaify *et al*., 2018).

3.1.3 Presence of chlorides, organochlorides, organophosphates and carbamates

All the three samples tested positive to chloride ion test. This can be attributed to the origin of the bio-slurry compounds which constituted of waste kitchen peels and inoculum.

Animal dung inoculum is a good source of chloride ions owing to several salts given to the animals (Thomas *et al.,* 2007). Sodium chloride is one such salt which is quite abundant in chloride ions. Interaction and complexation of this ligand with esters in the bio-slurry lead to formation of organochlorides (Supreeth and Raju, 2017). Such reactions are dependent on many factors thus only *Acanthaceae spp.* sample tested positive for organochlorides. **Table 3** below summarizes the presence or absence of chlorides, organochlorides, organophosphates and carbamates in the three pesticidal samples.

Table 3: Screening of presence of pesticidal groups in the samples

Pesticidal group	Sample		
	Control	*Terminalia b.*	*Acanthaceae spp.*
Chlorides	+	+	+
Organochlorides	-	-	+
Organophosphates	+	++	++
Carbamates	+	+	-

Pesticidal compounds screened in the bio-slurry samples

Key: + presence of compound

++ high amounts of compounds present

- compounds absent

All samples were quite rich in organophosphates. Bio-slurry is an excellent source of phosphorus thus organophosphates were abundantly formed. A good number of proteins both from the kitchen waste biomass and animal feed (as a precursor to inoculum) have appreciable amounts of phosphorus (Liu *et al.,* 2018). Degradation of this biomass in a biogas digester frees out phosphates which can easily combine with esters present to form organophosphates (Lorke *et al*., 2017). The control and *Terminalia b*. samples tested positive for carbamates. The findings are also supported by their IR spectra in 3.1.1 above. Carbamates result from disintegration of urea (Harvey, 2018). Animal dung (inoculum) is a good source of urea as urea is passed out as urine from most mammals (Beberashvili *et al.,* 2018). The stability of carbamates and urea in general are dependent on bacteria such as the *urease* bacteria which catalyzes the decomposition of urea to ammonia and bicarbonate ions (Sigurdarson *et al.,* 2018).

3.1.4 Total phosphorus, total phosphoric acid and free phosphates composition

Together with nitrogen phosphorus forms a good percentage of animal manure having originated from peptide bonds in the animal's feedstock (Liu *et al*., 2018). Phosphorus is also abundant in kitchen waste. However, unlike nitrogen which volatilize into the atmosphere as ammonia, or is reduced to other compounds such as nitrates by rhizobia bacteria, most phosphorus is not altered (Arbeli *et al.,* 2016). Phosphorus can exist as either elemental phosphorus in compounds such as phosphoric acid and other phosphates (Desianti *et al.,* 2017).**Table 4** below summarizes the concentrations of total phosphorus and total phosphoric acid content in the pesticidal samples.

Table 4: Total phosphorus and total phosphoric acid composition in the pesticidal samples

Parameter	Sample		
	Control	*Terminalia b.*	*Acanthaceae spp.*
Total Phosphorus (mg/L)	1.960±0.088	2.520±0.250	2.420±0.648
Total Phosphoric acid (mg/L)	2.963±0.333	3.477±0.023	2.771±0.231

Total phosphorus and phosphoric acid content in the bio-slurry samples

Terminalia b. samples were found to have high concentrations of both total phosphorus and total phosphoric acid of $2.520\pm0.250mg/L$ and $3.477\pm0.023mg/L$ respectively. Use of the additive enhance biomass degradability thus availing more phosphorus levels. Both forms are efficient in controlling pests. However, phosphoric acid is the better pesticide and a common fungicide (Silva *et al*., 2019). Total phosphorus is organic in nature and susceptible to biodegradability while total phosphoric acid is inorganic and quite durable (Weber *et al.,* 2000). However phosphoric acid is water soluble and can leach away to water bodies to cause water pollution (Beji *et al*., 2017). Some of the phosphorus exist as phosphates. This is the most suitable form of phosphorus for pesticide action (Harley *et al.,* 2016).Organophosphates represent a crucial part of these phosphates whereby the oxygen atoms in the compound are bound to organic groups (Lamichhane *et al*., 2019). Formation of organophosphates is an esterification process dependent on

several conditions such as temperature and pH (Christate *et al*., 2017). **Figure 2** below is a UV VIS spectrum illustrating the phosphates found in the three pesticidal samples analyzed.

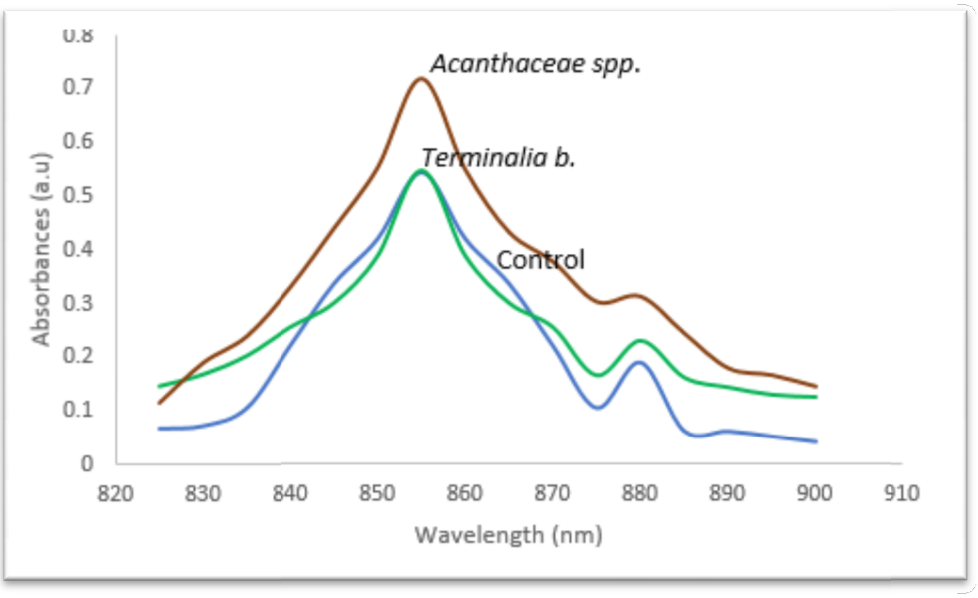

Figure 2; Phosphates in the pesticidal samples

From the above spectra, *Acanthaceae spp.* sample had more phosphate levels while both *Terminalia b.* and the control sample had almost similar compositions. The two additives, *Terminalia b.* and *Acanthaceae spp.* were thus found to increase the availability of all forms of phosphorus in the pesticidal samples.

3.2 Antimicrobial Analysis

Pesticides are known to have varying effects on microbial organisms. Some pesticides inhibit growth of microbes, others increase their growth rate while others have no effect (Seppala *et al*., 2007). The effects of the pesticidal samples on bacteria and fungi was analyzed and summarized in **table 5** below.

Table 5: Antimicrobial sample inhibition zones for the pesticidal samples

Microbe	Samples inhibition zone diameter (mm)		
	Control	*Terminalia b.*	*Acanthaceae spp.*
E. coli	17.000±0.500	16.000±0.000	14.000±0.000
S. aureus	12.500±0.000	16.000±0.000	14.000±0.000
C. albicans	10.000±1.500	15.000±0.000	6.000±0.000

Antimicrobial properties of the bio-slurry pesticidal samples

The variations in inhibition diameters of *E.coli* were found to be insignificantly different in the test samples ($p \leq 0.05$, n= 8).*E. coli* bacteria is specific to animals and barely survive on plants surface, not unless while feeding on plant exudates (Moreau *et al.,* 2019). Such survival is however quite short-lived (Weiss *et al*., 2019).High inhibition diameters recorded in the three samples implied that the pesticides were in a position to control these microbes from affecting the farmer. However, these results also imply that the plants sprayed with the extracts could offer solution to infected rodents and other mammalian pests. *S. aureus* bacteria were also significantly affected by the pesticidal samples. *Terminalia b.* sample recorded the highest suppression rate and was able to inhibit $16.000\pm0.000mm$ within 24 hours. Chlorine residues in pesticides are known to be particularly detrimental to gram-positive bacteria (Deshmukh *et al.,* 2016). The ions are able to penetrate the cell wall of the bacteria and destroy key cell organisms killing the bacteria (Dubey *et al*., 2004). Inhibition of *C. albicans* fungi was in the order of *Terminalia b*. ($15.000\pm0.000mm$), control ($10.000\pm1.500mm$) and *Acanthaceae spp.* ($6.000\pm0.000mm$). Fungi form a key part of plant pests notorious for killing plant parts and obtaining food from these parts (Nayan *et al*., 2019).

3.3 Efficacy Analysis

The viability of any pesticide lies on its efficacy in killing, suppressing or deterring pests. As a rule of thumb, a good pesticide should keep away pests. The test pesticidal samples were found to linearly kill aphids at a rate of2.083 for the *Acanthaceae spp.* sample and 1.670 for the control and *Terminalia b.* sample. However, fall armyworm efficacy analysis were not satisfactory as far as death of the pests was concerned. The rate of death and behavioral changes of fall armyworms upon exposure to the pesticidal samples were monitored for 48 hours and recorded in **table 6** below.

Table 6: Efficacy analysis of pesticidal samples on fall armyworm pests

Duration of exposure	Population and behavior	Sample			
		Efficacy tests control setup	Control	*Terminalia b.*	*Acanthaceae spp.*
0 hours	Population	10	10	10	10
	Behavior	Pests active	Pests active	Pests active	Pests active
6 hours	Population	10	10	10	9
	Behavior	Pests active	Activity reduce, no feeding	Activity reduce, no feeding	Activity reduce, no feeding
12 hours	Population	10	9	9	9
	Behavior	Pests active	Activity reduce, no feeding	Pests dormant, no feeding	Pests dormant, no feeding
18 hours	Population	10	9	9	8
	Behavior	Pests active	No feeding	No feeding	No feeding
24 hours	Population	10	9	9	8
	Behavior	Pests active	No feeding	No feeding	No feeding
30 hours	Population	10	8	9	8
	Behavior	Activity reduce but feeding normally	No feeding	No feeding	No feeding
36 hours	Population	9	8	8	7
	Behavior	Activity reduce but feeding normally	No feeding	No feeding	No feeding
42 hours	Population	9	8	7	7
	Behavior	Pests active	No feeding	No feeding	No feeding
48 hours	Population	9	8	7	7
	Behavior	Pests active	No feeding	No feeding	No feeding

Efficacy analysis of the pesticidal samples against Spodoptera frugiperda

Though the pesticidal samples did not deviate significantly from the death of the pests, there were notable changes in behavior of the pests. Movement of the pests in the test samples greatly reduced within a very short period. The pests coiled on exposure to the pesticidal samples and preferred the dry leaves without pesticidal residues. Feeding drastically reduced until the pests could no longer feed on the leaves anymore. Various botanical extracts have previously been used to suppress these pests with varying success levels (Mugwisi, 2017).

Unlike the fall armyworms, the aphids were rapidly killed and their population brought to zero within 30 hours. **Figure 3** below illustrates the rate at which the aphids were reduced by the pesticidal samples with time.

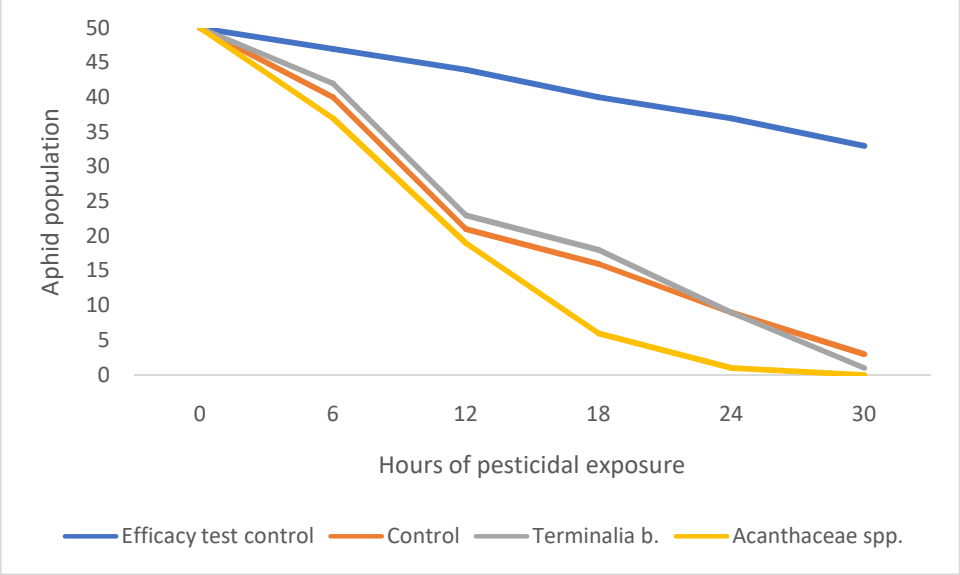

Figure 3; Reduction of aphids upon spraying with pesticidal samples

From figure 3 above, all the pesticidal samples were quite effective in reducing the population of aphids. Various types of organophosphate and carbamate have been successfully used to eliminate aphids (XIII International Entomophagous Insects Workshop, 2003).

3.4 Acute Dermal and Acute Irritational Toxicological Effects

All pesticidal samples were found out to be friendly to the user since there were no acute dermal and acute irritational toxicological effects. The specimen remained active all through the experiment period with normal feeding and other behaviors. The levels of acute irritation and acute dermal toxicology effect of the pesticidal samples on the test specimen are summarized in **table 7** below;

Table 7: Acute dermal and irritational toxicology effects of pesticidal samples

Toxicology effect	Type	Level of toxicity		
		Control	*Terminalia b.*	*Acanthaceae spp.*
Acute irritational	Oedema formation	0	0	0
	Erythema and eschar formation	0	0	0
Acute dermal	Dead specimen	0	0	0

Acute dermal and irritational toxicity of the pesticidal samples against test lab. animals

The pesticidal samples were found to be safe for use as far as dermal toxicity is concerned. Natural pesticides are usually safe due to biological compounds that buffer the effects of toxic pesticidal residues (Wang *et al*., 2019).

IV. CONCLUSION

The pesticidal samples had optimal physical-chemical and bio-chemical parameters to suit applicability, miscibility and durability in varying formulations. All samples were slightly acidic thus not susceptible for pesticidal alkaline hydrolysis. The variations in COD and BOD values were quite minimal as expected in most pesticides. The samples tested positive for crucial pesticidal compounds such as chloride ions and organophosphates. *Acanthaceae spp.* sample indicated traces of organochloride residue while both the control sample and *Terminalia b.* sample were positive in carbamates analysis test. Presence of these compounds was confirmed by halide, phosphide and carbamate peaks in their FT-IR spectra. Quantitative analysis indicated that while the *Terminalia b*. sample had more total phosphorus content, the *Acanthaceae spp*. sample had more total phosphoric acid and free phosphate content.

The pesticidal samples all tested positive for antifungal and antibacterial analysis. *Terminalia b.* sample portrayed the highest inhibition zone diameters of up to $16.000 \pm 0.000 mm$ for antibacterial tests and $15.000 \pm 0.000 mm$ for antifungal tests. The pesticides rate of killing fall armyworm was not satisfactory enough. The pesticides were only able to irritate the pests on touch, control their movement and prevent them from feeding. However, the samples successfully killed aphids within 30 hours of contact. The order of killing aphids was *Acanthaceae spp.* (2.803/hr) followed by *Terminalia b.* and control (1.670/hr for both). Acute dermal and acute irritational toxicology tests proved the pesticide sample to be dermally safe for use by farmers.

Both *Terminalia b*. and *Acanthaceae spp.* additives were found to increase the pesticidal properties of the bio-slurry samples.

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CONFLICTS OF INTEREST

The authors declare to have no conflict of interest whatsoever.

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