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Full Length Research Paper

Preliminary study on the effect of anaerobically digested cow dung slurry on the antimicrobial activity of three medicinal plants

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The study investigated the effect of anaerobically digested cow dung using polyethylene tube digester on the antimicrobial property of *Aloe barbadensis*, (Aloe vera) *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger). The methanol extracts of the three medicinal plants grown on soil augmented with anaerobically digested cow dung slurry exhibited marked antimicrobial activities on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Asperigillus niger* and *Trichophyton mentogrophyte* isolated from clinical specimens. All the three medicinal plants grown on digested cow dung slurry exhibited greater mean zones of inhibition on all the pathogens than that grown on soil augmented with undigested cow dung and soil not augmented at all. *A. sativum* (Garlic) raised on digested cow dung slurry exhibited the highest mean zones of inhibitions with spore germination of the fungi totally inhibited when compared with the two other medicinal plants. The results in this study have shown that anaerobically digested cow dung slurry does not only yield pathogen free manure, increased fertilizer and biogas but can improve the antimicrobial activity of medicinal plants.

Key words: Plastic digester, cow dung, anaerobic, soil, antimicrobial, medicinal plants.

INTRODUCTION

Antimicrobial agents have been used for over 70 years, since the synthesis of Penicillin by chain and Florey (1940), the development of antibiotics and antimicrobial agents is being intensified. Unfortunately, antibiotic resistance has sharply increased rendering many antibiotics ineffective. Besides the emergence of immuno-compromised cases and new strains of disease causing agents require that antibiotics are closely monitored (Kirby and Craig, 1981).

Apart from this, the search for new antimicrobial leads from plant sources as a possible alternative for antibiotic has gained widespread attention by many giant pharmaceuticals. Antimicrobial activity of medicinal plants has been reported by many investigators in Nigeria (Ebana et al., 1991; Okemo, 1996; Yongabi et al., 2000; Babayi et al., 2001) and in Kenya (East Africa).

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Related studies elsewhere, show that medicinal plants can be a valuable source of drugs if carefully harnessed. For instance in India, Kumar and Berwal (1998) reported the sensitivity of *Staphylococcus aureus, Escherichia coli* and *Listeria monocytogenes isolated* from processed foods to garlic (*Allium sativum*) extracts. While in Australia, a number of antimicrobial studies on oil extracts of *Melaleuca alternifolia* show promising activity (Lassak and McCarty, 1983; Mann and Markham, 1998.

In many developing nations, animal faeces have been composted and used to fertilize farm field (Ogbeide and Aisien, 2000; Audu et al., 2003). This has improved yield. More recently, slurry from anaerobic digesters have been used and found to condition soil and improve nutrient content and yield with out risk of disease transmission. We developed a hypothesis that anaerobically digested slurry could improve upon the antimicrobial activity of medicinal plants. Our purpose in this work is to report the effect of anaerobically digested slurry from cow dung liter

Plate 1. The anaerobic digester.

on the antimicrobial activity of three medicinal plants.

MATERIALS AND METHODS

Digester design and construction

The anaerobic digester used in this study was constructed using a longitudinal wooden trough(triangular in shape) the dimension of the trough were: Length of top and bottom 1.8 and 1.26 m respecttively while bottom width and top width, 0.45 and 0.4 m respectively according to methods described by An Bui et al. (1996) (plate 1).

At both ends of the trough, plastic pipes were initially cut to specifications using the Hawk saw and edges smoothened using the half round file. Fairly thick plastic polythene sheet with opening at both ends was first laid down as bedding in the trough and then folded two-step wise at both ends into layers and fed in gently through the pipes. The edges of the Polyethylene were wrapped round the mouth/opening of the pipes and fastened in position with a rubber band. The polythene sheet was thoroughly checked for holes so as to avoid any leakage when the process was fully operational.

Sample collection (cow dung)

The Cow dung were collected from the animal farm at Teaching and Research farm of the School of Agriculture, Abubakar Tafawa Balewa University, Bauchi using a spade and bucket as previously described by Theresa et al. (1993).

Slurry preparation

Two 10 L of buckets full of cow dung fresh was used. Slurry was made by mixing one part of the poultry liter in two parts of water. This was thoroughly mixed by stirring continuously for five min. A hundred litres of slurry was fed into the digester. As effluents were added to the digester, excess water drained off at the outlet. The digester was actually filled to the brim with space provision for gas to collect, although the major focus was to get the digested slurry (Yongabi et al., 2003).

Slurry sample collection for microbial analysis

When the slurry was fully made, three samples (10 mls each) were collected aseptically using sterile stainless steel spatula into sterile test tubes, the test tubes were sealed with cotton wool and then transported to the FMEnv/ZERI Research Microbiology Laboratory for analysis.

Similarly, the well water that was used in making the slurry, three samples of it were taken aseptically in the same way as described earlier for a comparative microbial analysis according to protocols previously described by Yongabi et al. (2003).

Microscopy analysis of slurries (raw and treated) and well water samples

Three smears of each of the samples were made on clean grease free slides and examined under the microscope using x100 and x400 magnifications respectively for protozoa, helminthes and observations recorded as described by Yongabi et al. (2003).

Microbial analysis of slurries (raw and treated) and well water samples

1 ml of the raw cow dung slurry was aseptically transferred into 9 mls sterile distilled water to give one in ten dilutions (10^{-1} dilution).The diluents were then serially diluted using 9 mls of sterile distilled water up to 10^{-3} dilutions. Using a sterile pipette, 1 ml each of 10^{-1}, 10^{-2} and 10^{-3} dilutions were carefully and aseptically inoculated in triplicates by the pour plate method (that is, 1 ml of the suspension mixed onto molten agar) onto salmonella shigella, nutrient, macconkey, eosin methylene blue agars for bacterial isolation and potato dextrose agar for fungal eosin methylene blue agars for bacterial isolation and potato dextrose agar for fungal isolation.

All the plates were incubated at 37°C for 24 h for bacteria and at 25°C for four days for fungal isolation. The above standard technique employed was adopted from Harrigan and McCance (1996). Three samples each were cultured and the same procedure was done for both the treated slurry and the well water samples.

Plate reading

Following incubation at 24 and 96 h respectively for bacteria and fungi respectively, the plates were read off. The cultural characteristics such as shape of colonies, colour, etc were observed macroscopically and recorded. Then a discrete bacterial colony from each plate was gram stained and observed microscopically at 1500 magnification according to the method of Cheesbrough (1984). Equally, discrete fungal colonies were observed microscopically in a Lactophenol cotton blue preparation.

The cell morphologies as well as unique differential features were recorded. The characteristics features collated were compared with taxonomical keys specified in Bergey's manual of determinative Bacteriology (Buchanan and Gibbons, 1980) to give an identity to the bacterial isolates while the keys specified in Barnett and Hunter (1972) were used to identify the fungal Isolate.

Microbial analysis of treated slurry

Anaerobic digestion of the raw slurry was allowed for five weeks at mesophilic temperature. Following this treatment and as the gas start to fill in the gas collector, three samples of the slurry now (treated slurry) were aseptically collected and analysis followed the same procedure as earlier described (Yongabi et al., 2003).

Similarly, it was at this time that the well water samples which were initially used in making the slurry and kept at room temperature 37°C in the laboratory for five weeks were then microbiologically analyzed in the same procedure.

pH analysis

The pH of the raw and treated slurries were tested using a combi-9 test strip (a standard strip for routine urinary biochemical analysis) the strip was dipped into the slurries and after 60 s, the colour change noticed was compared with a range of colour standards and when the colour of the strip matched any of the colours, the pH value was directly read off (Yongabi et al., 2003)

Source, identification and processing of plants materials

The plants; *Aloe barbadensis* (Aloe Vera), *A. sativum* (Garlic) and *Zingiber officinale* (ginger) were cultivated at the research farm of school of Agriculture, Abubakar Tafawa Balewa University by undergraduate student studying the effects of cow dung on the yield of a number of crops. We set up plots of 5 m^2 each of the three medicinal plants and cultivated each of them on soil without any fertilizer, on soil fertilized with cow dung (undigested) and a third plot on soil fertilized with digested poultry liter (slurry) from the plastic anaerobic digester we had set up 8 weeks earlier at the FMEnv/ZERI Research Centre (Babatunde and Yongabi, 2008).

All agronomic/horticultural care in terms of weeding etc was given. The experiment was done in the rainy season June to October. In October, 2005, samples of each of the plants from each of the plots were harvested and rinsed a couple of times in clean water then peeled using a knife. The fresh plants were pounded using a pestle and Mortar.

Extraction procedures

50 g of each of the Marsh was steeped in 200 mls of 95% methanol (BDH Chemical poole, Uk) in Erlenmeyer flasks and allowed to extract for 72 h. The extracts obtained were passed through a whatman filter paper No. 1 (whatman, Uk) and concentrated in vacuo using a rotary evaporator (Buchii laboratory Technique, Switzerland at 37°C. The extracts were then stored in sterile screw capped bottles and kept in the refrigerator for the bioassay test (Yongabi et al., 2000).

Test bacteria and fungi used in this study

The bacterial isolates used in this study were *S. aureus, E. coli* and *Pseudomonas aeruginosa* isolated from patients with antibiotic resistant urinary tract infections at the University Clinic, ATBU, Bauchi, Nigeria. While the test fungi *Asperigillus niger* and *Trichophyton mentagrophyte* were isolated from a case of *Tinea capitis* attending the above clinic.

Determination of antibacterial activity of plant extracts

The agar diffusion method as described by Collins et al. (1995) was used. 0.2 ml each of the plant extract was filled into wells 6 mm in diameter that were aseptically drilled using a cork borer. 18 h peptone water grown culture of the test organisms was inoculated in the plates by the pour plate techniques and allowed to solidify prior to this. The extracting solvent (methanol) 0.2 mls of it was also introduced into a well as control.

The plates were incubated at 37°C for 24 h. The development of zones of inhibition around the holes containing the extract indicated the antimicrobial activity of the plant extract against the test organisms. The difference between the zones of inhibition observed for the test and that of the control was recorded as the actual diameter of zone of inhibition caused by the plant extract.

Determination of *in vitro* antifungal activity of the extracts

The test fungi were previously isolated from the scalp of patient presenting with *T. capitis* at University Medical Centre, ATBU. Bauchi, Nigeria and isolates maintained on sabauraud dextrose agar (SDA) slants (Difco). The Methanol extract of each of the plants was diluted with assay media (SDA) 0.5 ml of the extracts each was mixed homogenously with 19.5 ml of SDA and allowed to solidify.

All set ups were done in duplicate and a control plates with no extracts was raised. A uniform portion of the test fungi was removed using a 5 mm steel borer and aseptically placed onto a 5 mm well drilled on the assay media. Diagonal lines were initially ruled using a bold marker on the back of the agar plate to ease measurement of mycial spread. The set up was carefully sealed all around with a sticker tape to avoid any aerial contaminants and carefully incubated at room temperature 25 - 37°C for 10 to 3 weeks. The radial mycelium spread was measured using a vernier caliper on the 10^{th} and 21^{st} day and mean values recorded. This was done according to protocols described by Yongabi et al. (2000).

RESULTS AND DISCUSSION

The bacteriological analysis of the raw and anaerobically digested cow dung showed a significant difference in bacterial counts with lower counts for the anaerobically digested slurry (Table 1). This has shown that plastic anaerobic digester is efficient in decontaminating cow dung and may also be useful in decontaminating water as shown (Table 2). The finding is consistent with an earlier observation by Yongabi et al. (2003)

The mean antibacterial effect of Aloe vera, garlic and ginger cultivated on soil fertilized with anaerobically digested slurry is shown in Table 3. The mean inhibitory zones of digested slurry are greater than with those observed from the same plants grown on soil fertilized directly with undigested cow dung and as well as on soil without fertilizer at all (Tables 4 and 5) (Plate 2).

Generally, all the plant extracts exhibited activities on the test organisms proving that their medicinal potentials as reported in previous studies (Kumar and Berwal, 1998; Yongabi et al., 2002). The finding in this study has proven that there is enhanced antibacterial activity when these

Table 1. Bacteriological analysis results of raw and treated cow dung slurries at 10^{-3} dilution.

	Total aerobic mesophilic counts	coliform counts	E. coli counts	SS counts	Yeast count
Raw slurry	TNTC	TNTC	TNTC	TNTC	TNTC
Treated slurry	44 CFU/ml	8 CFU/ml	2CU/ml	Nil	13 CFU/ml

TNTC: Too numerous to count; CFU/ml: Colony forming unit per mililitre; Nil: No colonies isolated; *E. coli*: *Escherichia coli*; SS: *Salmonella* and *shigella*.

Table 2. Bacterial analysis result of well water and raw cow dung slurries placed on bench for 3 weeks at 10 - 3 (well water from university campus).

Total aerobic mesophilic counts	Coli form counts	*E. coli* counts	ss counts	Yeast count
At the beginning of experiment TNTC	TNTC	TNTC	Nil	TNTC
After 5 weeks of bio-digestion TNTC	5.2×10^4	3.7×10^4	Nil	TNTC

TNTC - Too numerous to count.
Nil - No isolates.

Table 3. Mean antibacterial effect of *Aloe barbadensis, Allium sativum and Zingiber officinale* cultivated on soil fertilized with cow dung anaerobically digested slurry in mm.

Medicinal plant / Spice plant	Diameter zone of inhibition		
	S. aureus	***E. coli***	***Pseudomonas* sp.**
Aloe barbadensis (Aloe vera)	23	19	14
Allium sativum (Garlic)	29	30	18
Zingiber officinale (Ginger)	19	28	14

Table 4. Mean Antibacterial activity of *Aloe barbadensis*, *Allium sativum* and *Zingiber officinale* raised on soil with direct cow dung liter in mm.

Medicinal plants / Spice plant	*S. aureus*	*E. coli*	*P. aeruginosa*
Aloe barbadensis	11	8	10
Allium sativum	19	17	15
Zingiber officinale	10	12	10

Table 5. Mean Antibacterial activity of Methanol extracts *A. barbadensis, A. sativum and Z. officinale* grown on soil without fertilizer (control) in mm.

Medicinal plant/Spice plant	*S. aureus*	*E. coli*	*P. aeruginosa*
Aloe barbadensis	14	11	8
Allium sativum	17	16	13
Zingiber offinale	8	10	7

plants are grown on soil fertilized with cow dung and greater antibacterial activity when digested slurry is used. Previous agronomic studies showed that the nitrogen content of crops is a function of the type and quantity of fertilizer used (Greenwood, 1982).

The three plants inhibited growth of *T. mentagrophyte* and *A. niger* at varying degrees (Tables 6 - 11) and better than controls. Apart from the fact that these plants exhibit antifungal activity, the effect of anaerobically digested cow dung slurry thus appear to enhance their antifungal activity.

This finding probably lends credence to why many native herbalists prefer to harvest their medicinal plants on rich organic soil. The possibility that the slurry may be adding the quantity of the bioactive ingredients in these plants cannot be ruled out. In an unrelated study, Mahran

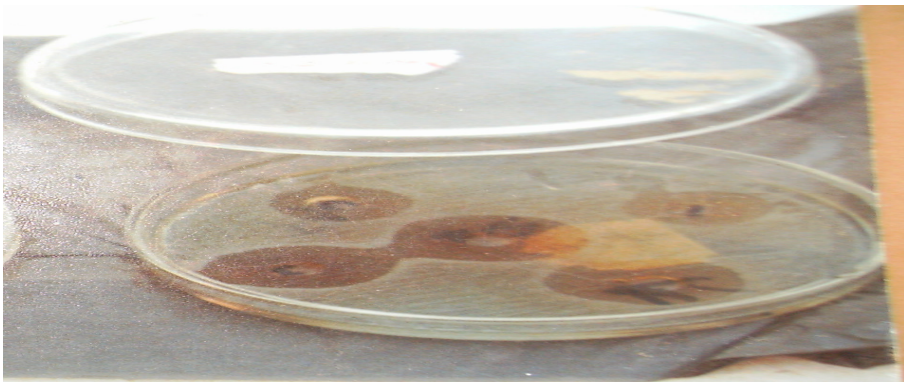

Plate 2. A plate showing marked antibacterial activity of *A. sativum* grown on anaerobically digested slurry on *P. aeruginosa*.

Table 6. Mean Antifungal activity of methanol extracts of *A. barbadensis, A.sativum and Z. officinale* grown on soil fertilized with anaerobically digested cow dung on *A. niger.*

Medicinal plant/ Spice plant	Spore germination (after 10 days)	Radial mycelia spread in mm (in 10 days)
Aloe barbadensis (Aloe vera)	germination-	6.0
Allium sativum (Garlic)	No germination at all	-
Zingiber officinale (Ginger)	germination	10.0
Aspergillus niger plate	Full germination	21

Table 7. Mean antifungal activity of methanol extracts of *A. barbadensis, A. sativum and Z. officinale* grown on soil fertilized directly with cow dung liter on *A. niger.*

Medicinal plant/ Spice plant	Spore germination after 10 days	Radial mycelial spread in mm in 10 days
Aloe barbadensis (Aloe vera)	Germination	8
Allium sativum (Garlic)	No germination	-
Zingiber officinale (Ginger)	Germination	10
Aspergillus niger control plate	Full germination	21

Table 8. Mean antifungal activity of methanol extracts of *A. barbadensis, A. sativum* and *Z. officinale* grown on soil without fertilizer on *A. niger.*

Medicinal Plant/ Spice plant	spore germination	radial mycelia spread in mm (in 10 days)
Aloe barbadensis (Aloe vera)	Germination	11.5
Allium sativum (Garlic)	No germination	-
Zingiber officinale (Ginger)	Germination	14.2
Aspergillus niger control plate	Full germination	21

Table 9. Mean Antifungal activity of Methanol extracts of *A. barbadensis*, *A. sativum* and *Zingiber officinale* grown on soil fertilized with anaerobically caw dung digested slurry on *T. mentagrophyte* in mm.

Medicinal plant/ Spice plant	Spore germination	Radial Mycelia spread in 3 weeks
Aloe barbadensis (Aloe vera)	Germination	9
Allium sativum (Garlic)	No germination	0
Zingiber officinale (Ginger)	Germination	15
Trichophyton mentagrophyte control culture plate	Full germination	26

Table 10. Mean antifungal activity of methanol extracts of *A. barbadensis*, *A. sativum* and *Z. officinale* grown on soil fertilized directly with cow dung liter on *T. mentagrophyte* in mm.

Medicinal plant/ Spice plant	Spore germination	Radial mycelial spread in 3 weeks
Aloe barbadensis (Aloe vera)	Germination	12.4
Allium sativum (Garlic)	No germination	0
Zingiber officinale (Ginger)	Germination	13.5
Trichophyton mentagrophyte control culture plate	Full germination	26

Table 11. Mean antifungal activity of methanol extracts of *A. barbadensis*, *A. sativum* and *Z. officinale* grown on soil without fertilizer on *T. mentagrophyte* in mm.

Medicinal plant spice	Spore germination	Radial mycelia spread in 3 weeks
Aloe barbadensis (Aloe vera)	Germination	14.2
Allium sativum (Garlic)	No germination	0
Zingiber officinale (Ginger)	Germination	18
Trichophyton mentagrophyte control culture plate	Full germination	26

et al. (1978) reported that, when *Hibiscus sabdariffa* was grown on soil with additional nutrients, the growth and yield was significantly enhanced.

The bacterial isolates used in this study were from patients who have urinary tract infections and have used a panapoly of antibiotics with no significant benefits and specimens were now sent to the laboratory for comprehensive antibiogram tests. From our findings, possible isolation of the bioactive ingredients from these plants when cultivated on anaerobically digested cow dung slurry can play a great therapeutic role in treating antibiotic resistant urinary tract infections.

Similarly, the antifungal resistance to imidazole derivatives is widespread in clinical practice today especially in Africa with the rising incidence of immunosuppressive diseases such as HIV and cancer. Our research findings equally indicate that, an antifungal drug could be developed from medicinal plants cultivated on the bye product of a bioconversion process of cow dung using plastic digesters. It's amazing that cow dung can be disinfected through such a process and then biogas produced as a clean fuel option and biofertilizer to boost agriculture and medicine.

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