

# Microbial and Physicochemical Properties of Fermented African Locust Bean (*Parkia biglobosa*) Effluent and its Biocidal Potential on some Selected Insects

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**Abstract:** The study was carried out to determine the microbial and physicochemical identify the bacteria and fungi associated with the fermentation of African Locust Bean (*Parkia biglobosa*) “Iru” effluent and to assess the biocidal effect of fermented *Parkia biglobosa* effluent on some selected insects (mosquito, bean and rice weevils). The physicochemical properties of the fermented locust bean effluents were assessed. The results obtained on the effluent physicochemical parameters showed that there were differences between the control effluent and the fermented African locust bean effluent from the laboratory. Specifically, total hardness (614.00 – 194.00 mg/l), total dissolved solids (102.15 – 108.53 mg/l), total solids (125.30 - 131.50mg/l), the total alkalinity (762.50 – 688.75 mg/l) and the total acidity (as CaCO<sub>3</sub>) (206.70 – 263.40 mg/l). The minerals of the two effluents showed sharp differences in the sodium, potassium, calcium and magnesium composition. Control effluent was higher than the test effluent in sodium (1578.19 mg/l), potassium (4734.19 mg/l) and magnesium (4304.16 mg/l) while the test effluent was only higher in calcium (7259.53 mg/l) compared to that of the control. The microbial types in fermented samples were identified after the fermentation period of 72 hrs. The bacterial profile were *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Staphylococcus aureus*, *Leuconostoc mesenteroides*, *Escherchia coli* and *Enterococcus faecalis*. *Leuconostoc mesenteroides* and *Bacillus subtilis* had the highest occurrences. The fungi isolates were *Fusarium* sp., *Saccharomyces cerevisiae*, *Aspergillus fumigatus*, *Aspergillus niger* and *Penicillium* sp. Evaluation of the effect of fermented African locust bean effluent on bean weevil *Callosobruchus maculatus* (F.) with respect to insect mortality showed considerable biopesticidal potential on the bean weevil. The results revealed that the fermented locust bean effluent had the highest mortality of six after 20 minutes and the lowest mortality of one after 40 minutes. With further research, fermented African locust bean effluent can be used as biopesticide which would be cheaper, less hazardous to human health and easily accessible to local people to preserve cowpea produce.

**Keywords:** Biocidal Effect, Bean Weevil, Fermentation, Effluent, *Parkia biglobosa*

## Introduction

There has been growing concern on the methods of processing most agricultural products due to their adverse impact on the environment causing pollution and also affect lives. Some research have been carried out on the production of fermented condiments, ‘iru’ from African locust bean (*Parkia biglobosa*) (Odunfa, 1986); melon seed fermented ogiri (Odunfa, 1981) and soybean produced dawadawa (Omafuvbe *et al.*, 2000, 2002). This African fermented condiment requires food processing technologies that will meet the requirement of human needs. Fermentation as one of the methods of processing agricultural products improves the digestion, nutritive value, and flavour of

the raw materials used (Ogunshe, 1989; Ogunshe *et al.*, 2006). Traditional fermentation processes used in the production of these foods are uncontrolled and are dependent on microorganisms from the environment or the fermentation substrate for initiation of the fermentation processes. Microorganisms and metabolic pathways associated with the production of fermented foods are the subject of considerable research, targeting strain isolation and identification; improvement of the efficiency of fermentation processes and the quality, safety and consistency of fermented foods (Tietyen *et al.*, 2000; FAO, 2004). The fermentation of African locust beans (*P. biglobosa*) by *Bacillus* spp. to produce ‘iru’ via alkaline fermentation has been

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discussed by several workers in West Africa (Odunfa, 1986) and in India. Microbial fermentation of locust bean have been found to involve only bacteria since fungi found have been regarded as incidental and does not play any notable role in its fermentation (Ikenebomah and Ingram, 1986). Bacteria implicated are facultative anaerobes, while approximately 10% are aerobic after 36 hrs of fermentation. It was also observed that in some Nigerian fermented samples, *Pediococcus* and two varieties of *Staphylococcus saprophyticus* were detected. Several other workers have also implicated *Bacillus subtilis* in the alkaline fermentation of locust beans (N'dir *et al.*, 1997; Ouoba *et al.*, 2002; Ouoba *et al.*, 2003). Campbell-Platt (1980) also mentioned lactic acid bacteria especially species of *Lactobacillus* and *Pediococcus* as partakers in the fermentation of locust beans that gives characteristic properties to the final product.

Locust bean effluent, a waste product from fermentation process of African locust bean can be harmful to pest, aquatic life and other animals. Regardless of its taste and offensive odour, it is a major source of protein, oil, carbohydrate, vitamins and minerals (Campbell-platt, 1980). This effluent can be of economic values such as protein and nutrient source for animal feeds, biofuel production and may serve as a good biopesticide.

Controlling stored pests is not an easy job, although synthetic chemicals are available for use. Effective pest control is no longer a matter of heavy application of pesticides (because many rural area farmers resulted to the use of large quantity insecticides because of lack of application knowledge), because excessive use of pesticide promotes faster evolution of resistant form of pests, destroys natural enemies, turns formerly innocuous species into pests, harms other non target species and contaminates food (Busungu and Mushobozy, 1991). There is, thus, an urgent need for control agents, which are less toxic to man and more readily degradable. It had been well reported that extracts from a variety of plants have potent insect pest-control properties, and they have been found to affect the biology of target insects in different modes such as ovicides, repellents, fumigants, contact toxicants, and insecticides (Sen, 2001). In addition, plant-based pesticides are renewable in nature and cheaper. Also, some plants have more than one chemical as active ingredients responsible for their biological properties. These may be either for one particular biological effect or they may have diverse ecological effects. The chances of developing quick resistance to different chemicals are highly unlikely (Saxena *et al.*, 1989).

Bean weevil is harmless to people, houses, furniture, clothing and pets. It doesn't bite or sting and do not

carry diseases. The harm it does is destruction of the seeds they infest (Casey, 1994). Over 90% of the insect damage to cowpea seeds is caused by the "cowpea weevil" *Callosobruchus maculatus* F., a pest to several pulses including chickpeas (*Cicer arietinum* L.), lentils (*Lens culinaris* Medik.), soybeans (*Glycine max* Mer.) and common beans (*Phaseolus vulgaris* L.). Indeed *C. maculatus* infestation on stored legumes may reach 50% within 3 to 4 months of storage. Metabolic activity generates heat and produces water through the process of condensation, which encourages mold growth and grain spoilage (Magan *et al.*, 2003). Subsequent infestations in store result from the transfer of infested grain into store or from the pest flying into storage facilities, probably attracted by the odour of the stored grain. In stored beans, heavy infestation of this pest may cause weight losses of as much as 30 to 40 %, although losses are commonly 4 to 5 % (Casey, 1994). The chewing damage caused by the weevils, brings about increased respiration in the seeds, which promotes evolution of heat and moisture and in turn provides favorable living condition for molds leading to production of aflatoxin. Subsequently, at very high moisture levels, bacterial growth is favored which ultimately gives rise to depreciation and finally total loss (Dahiya, 1999).

Unlike other stored product insects, adults of this beetle can be found in two morphological body forms: one with wings and capable of flight and the other without wings and flightless. The flying form is produced when larval rearing conditions are crowded, or in continuous light or dark (such as in storage), high environmental temperature, or low moisture content (Beck and Blumer, 2011), conditions often found in storage. In storage, the flightless form is common. As the population grows and conditions become unsuitable, the winged form appears and disperses to breed on growing seeds in the field. Adults often are found in the field on flowers in the spring. Winged females oviposit on beans in the field and the resulting larvae are transported into storages at harvest (Arbogast, 1991). Females of the flightless form lay more eggs, and those eggs have a different hatchability than females that fly. Egg hatchability increases from 45.9 to 64.1 % for flying versus flightless females at 15 °C but decreases from 22.5 % (flying) to 1.8 % (flightless) at warm temperatures at 35 °C. Flying form females emerge with immature ovaries and oviposition is delayed three to four days. They withstand cooler temperatures and require higher humidity. Flying form adult longevity is twice as long as the flightless form (Utida, 1972).

Fecundity depends on the host, with poor oviposition on lentils (23 eggs per female) to optimal oviposition on broad beans (110 eggs per female) (Utida, 1972). Females lay eggs on the outside of the seed and

newly emerged larvae bore inside, multiple larvae inhabiting a single seed. Larvae are white and c-shaped. Damage occurs due to larval feeding. Larvae burrow into the seed and feed on the embryo and endosperm until pupation (Utida, 1972).

Identification of microorganisms responsible for the fermentation process of Africa locust bean is of great

importance for best fermentative product. The effectiveness of fermented Africa locust bean effluent as possible cowpea protectant and biopesticide on bean weevils (effectiveness of this effluent was based on the adult mortality tests under laboratory conditions). The possibility of the effluent serving as alternative to synthetic chemicals in rural areas was one of the impetuses and main focus of this research.



**Plate 1: African locust bean seed**



**Plate 2: A Bean weevil** (Source: Linsley and Michelbacher, 1943)

## Materials And Methods

### Collection of samples

The African locust bean effluent used for this study were labeled; sample A and B. Sample A was prepared in the laboratory and sample B was obtained from Oja- oba market, Akure, Ondo State (7°10'N 5°05'E), Nigeria. The grains were screened; broken

ones' and those perceived to be of low quality were removed.

Africa Locust bean seeds were boiled for 12 hours to soften the hard testa after which they are de-hulled by hand. The separated cotyledons are boiled for another 2 hrs to soften them. About 20 g of the seeds, which

are in good condition were cooked, de-hulled and parboiled in an aluminum pot. It was aseptically transferred to a sterile container, covered and allowed to undergo fermentation. The effluent collected from washing off the pod and pod removing process known as de-hulling of the seed was labeled as sample A. The effluent obtained from Oja-oba market, Akure was labeled sample B. Sample B served as the control. The locust beans effluents were then fermented for 72 hours in the laboratory. The container containing the fermentation effluent was aseptically accessed each time sampling was done to prevent termination and contamination of the fermentation process. The effluent samples were subsequently analyzed microbiologically and for biopesticidal effect.

### **Biocidal test of effluent on bean weevil**

#### **Rearing of weevil**

Adult bean weevil [*C. maculatus* (F.)] was isolated from already infested cowpea [*V. unguicalata* (L.)] obtained from the Research Laboratory of the Department of Crop, Soil and Pest Management, FUTA (7.2995° N, 5.1471° E), Ondo State, Nigeria. Cowpea used to culture the weevil was thoroughly cleaned and exposed in an oven to ensure the absence of insects, mites or disease-causing microorganisms. The treated cowpea seeds were then put inside rubber containers previously washed, sterilized and dried. Into the container containing cowpea seeds was added bean weevil obtained from infested bean grains. The plastic containers were covered with a net fastened tightly with rubber bands. The rearing of the insects was done in the laboratory to adapt them to the laboratory conditions of 28°C ± 2°C and 70 ± n5% R.H., with a 14:10 (L:D) photoperiod. The rearing was given enough time until new adult insects emerged. These were then used for the experiment.

#### **Adult mortality test**

5mls each of the fermented African locust bean effluents was measured into a sprayer to be used. Ten bean weevils were placed into a clean sterile container and were labeled. Few uninfested cowpea were placed at the centre of the containers. To the other control container was also added ten active adult bean weevils (*C. maculatus*) but was not sprayed. The containers were then sprayed with the fermented African Locust bean effluent and were monitored. The containers were then loosely covered to allow passage of air. Weevil mortality was assessed and recorded after 10, 20, 30 and 40 minutes of applying the effluents.

#### **Sample preparation and isolation of microorganisms**

One ml of each fermented locust beans effluents (sample A, B) was diluted with 9ml of sterile distilled water to make the stock solution and the progressive

serial dilution of stock solution of 10<sup>-1</sup> to 10<sup>-5</sup> was done separately for each sample. The solution (0.2 ml) was pipetted into labeled sterile Petri dishes, after which it was cooled. The molten agar media was poured on it and swirled gently for even distribution of bacteria and fungi in the plate. Then the plates were allowed to set or solidify and subsequently incubated upside down to avoid condensation for 24 hours at 37 °C for bacteria and at 25 °C for 48 hours for fungi plate. After incubation, the plates were observed for bacterial growth and fungal growth respectively.

#### **Identification of fungi**

A drop of cotton blue in lactophenol was placed on a clean grease free microscope slides and then a tiny mycelium was taken from the sub-cultured plate with the aid of an inoculating needle or loop and placed on a clean grease-free slide to make a smear. The slide was covered with a cover slip and examined under the microscope with ×10 objective lens for the morphological and microscopic structures. The fungi isolate were also characterized based on the colour of the colony and vegetative parts (Samson *et al.*, 2002).

#### **Biochemical and morphological identification of bacterial isolates**

Individual colonies were identified by morphological and biochemical techniques using Holt *et al.*, (1994), Cheesebrough, (2006), Fawole and Oso, (2007).

#### **Physico-chemical analysis**

The pH was measured using pH meter standardized at pH 7.0 using appropriate buffers (Hendershot *et al.*, 1993). Biochemical oxygen demand was determined using the method described by APHA (2005). The moisture content of each soil sample was determined by drying 10grammes of the soil in an oven at 80 °C until a constant weight was reached and the percentage moisture content was calculated. The organic carbon content was determined using the Walkley-Black wet oxidation method as described by Ibitoye (2006). Available phosphorus, chloride, sulphate, exchangeable bases (magnesium, calcium, sodium and potassium ion) concentration were determined using standard methods (AOAC, 2012). Total nitrogen was measured using the kjeldahl digestion method (Heads, 1992) and the heavy metals determination were estimated using atomic absorption spectrometer (ASS). The other physiochemical parameters determined using standard procedures as described by AOAC, 2012 were temperature, turbidity, total hardness, alkalinity, total solids, total suspended solids (non-filterable solids) and total dissolved solids.

## Results

### Microorganisms identified from the fermented African locust bean effluent

Fungi isolated and identified from the fermented locust beans effluents in the laboratory were *Fusarium* sp., *Saccharomyces cerevisiae*, *Aspergillus fumigatus*, *Aspergillus niger* and *Penicillium* sp. while fungi from fermented African locust beans effluent collected from the market were *Fusarium* sp., *Saccharomyces cerevisiae*, *Aspergillus fumigatus*, *Aspergillus niger*, *Trichoderma* spp and *Penicillium* sp.

The bacteria identified from fermented locust bean effluent in the laboratory were; *Bacillus subtilis*, *Bacillus cereus*, *Leuconostoc mesenteroides*, *Staphylococcus aureus* and *Enterococcus faecalis*. They were all gram positive and they were all glucose and sucrose fermenters.

Bacteria from fermented African locust beans effluent collected from the market were *Leuconostoc mesenteroides*, *Bacillus subtilis*, *Proteus mirabilis* and *Escherchia coli*. *Leuconostoc mesenteroides* and *Bacillus subtilis* were common to all the fermented African locust bean effluents.

**Table 1: Physicochemical properties of the fermented locust bean effluents**

Parameters	Sample A	Sample B
Temperature (°C)	25.50°C	26.25°C
Colour	Black	Brown
Odour	Objectionable	Objectionable
Turbidity (NTU)	1.20	1.20
pH	6.71	6.55
Total solids(mg/l)	125.30	131.50
Total suspended solids(mg/l)	23.15	22.98
Total dissolved solids (mg/l)	102.15	108.53
Total hardness(mg/l)	614.00	194.00
Total alkalinity (mg/l)	762.50	688.75
Total acidity ( as CaCO <sub>3</sub> ) (mg/l)	206.70	263.40
Dissolved oxygen(mg/l)	2.33	2.61
Biochemical oxygen demand(BOD)(mg/l)	0.25	0.20

**Key:** Sample A= the fermented effluent prepared in the laboratory (LE)  
Sample B= the fermented effluent collected from the market (ME)

**Table 2: Mineral composition of the fermented locust bean effluents**

Minerals	Sample A	Sample B
Chloride (mg/l)	0.00	0.00
Nitrate (mg/l)	2.12	1.71
Sulphate (mg/l)	2.99	2.88
Phosphate (mg/l)	100.16	110.27
Sodium (mg/l)	771.32	1578.19
Potassium (mg/l)	589.84	4734.19
Calcium (mg/l)	7259.53	5451.94
Magnesium (mg/l)	2177.89	4304.16
Zinc (mg/l)	36.30	54.52
Iron(mg/l)	51.72	50.22
Copper(mg/l)	7.26	2.87
Nickel(mg/l)	0.00	0.00

**Key:** Sample A= the fermented effluent prepared in the laboratory (LE)  
Sample B= the fermented effluent collected from the market (ME)

### Result of the test carried out on beans weevils

Fermented locust bean effluent A and B affected the bean weevils; they were initially weak after spraying and the mortality rate were recorded and presented in table 3;

**Table 3: Mortality test result**

Time (Minutes)	Number of weevil kill by sample A	Number of weevil killed by sample B
10	0 (weak weevil)	0 (weak weevil)
20	6	6
30	2	3
40	2	1

**Key:** Sample A= the fermented effluent from de-hulling of the locust bean  
Sample B= the fermented effluent collected from Oja-oba market Akure

### Discussion

The physicochemical properties of the fermented locust bean (FALB) effluent showed that the effluent might be of negative health implications to human and other living cells if it is continually released to the environment. The parameters showed that there were differences between the control effluent and the FALB effluent from the laboratory. Specifically, total hardness (614.00 mg/l), total dissolved solids (102.15 mg/l), total solids (125.30 mg/l) (Table 1), which might have depended on the type of water used, boiling time, temperature of boiling, the constituent of materials boiled, while the total alkalinity (762.50 mg/l) and the total acidity (as CaCO<sub>3</sub>) (206.70 mg/l) variations could be due to differences in boiling time, temperature of boiling, the constituent of materials boiled, length of fermentation and the types of microorganisms involved in the fermentation of the effluents.

The mineral compositions of the two effluents showed sharp differences in the sodium, potassium, calcium and magnesium composition. Market effluent (ME) was higher than the laboratory effluent (LE) in sodium (1578.19 mg/l), potassium (4734.19 mg/l) and magnesium (4304.16 mg/l) while the laboratory effluent was only higher in calcium (7259.53 mg/l) compared to that of the ME (Table 2). These differences could also be due to differences in source or species of locust bean, boiling time and temperature, length of fermentation and the types of microorganisms involved in the fermentation of the effluents.

The bacterial and fungal diversity of African locust bean effluents shows that the frequency of *Bacillus subtilis* and *Leuconostoc mesenteroides* occurred in both effluent samples. This could be a pointer to their versatility in nutrient usage and their importance in the fermentation of African locust beans. Nutrient usage versatility and adaptation to different environments of *Bacillus subtilis* had been documented (Ashlee *et al.*, 2008; Krijgsheld *et al.*, 2013). The preponderance of *Bacillus* species (*Bacillus* species isolated from various plant protein sources have also been reported to be proteolytic and are able to break down oils) have been reported in other fermenting legumes (Omafuvbe *et al.*, 2002).

Studies by Antai and Ibrahim (1986) and Odunfa (1985) had shown that several microorganisms are associated with locust beans fermentation and noted that the most abundant and the major dominant agent of fermentation after 24hrs was *Bacillus subtilis*. They also noted the presence of *Leuconostoc mesenteroides* and *Staphylococcus* species after fermentation. Similar report also showed that *Bacillus subtilis*, *Leuconostoc mesenteroides* and *Staphylococcus* sp were associated with the fermentation of African locust bean seeds to produce 'iru' condiment (Odunfa, 1981; Omafuvbe *et al.*, 2002). *Staphylococcus* species have been found to be associated with fermenting foods of plant origin particularly vegetable proteins. Presence of *Staphylococcus* could also have been caused by handling of the seeds after boiling (Omafuvbe *et al.*, 2002; Quinn and Cole, 2007). The presence of *Escherichia coli* indicate possible faecal contamination, which could be from the water used for de-hulling the locust bean effluent. *Escherichia coli* and *Enterococci* have been associated with human and animal faeces, it can also be found in sewage, untreated effluents, and all natural waters and soils subject to recent faecal contamination, whether from humans, wild animals, or agricultural activities. The presence of other microorganisms in African locust bean effluent also indicated their involvement in the fermentation processes. Probably they can use the nutrients present in these effluent producing fermented product and possibly toxic materials which might be harmful to cells or lives that is exposed to it. These toxic products may be responsible for the biopesticidal potential of the effluent.

*Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp. were present in the effluent. These fungi were observed to be the major fungi associated with locust bean fermentation and this is in accordance with Oladunmoye, (2007) who studied the effects of fermentation on nutrient enrichment of *Parkia biglobosa* and observed that the species of *Penicillium*, *Aspergillus* and *Fusarium* are the fungi species that are involved in the fermentation of locust beans. Their present could also be due to the fact that the soil contains a wide range of microorganisms including fungi which might have been transferred to the seeds during collection (Rodriguez and Redman,

2008).

The insect mortality test of the two effluents (LE and ME) on bean weevil showed that both effluents had adverse effect on bean weevil (Table 3). This could be as a result of harmful or toxic fermentative products in the effluents. Toxicity is defined as the measure of how poisonous a substance is or how large a dose is required to kill or change an organism (ECETOC, 1985). It has been reported that the locust bean husk combined with cassava peelings can be used to control root rot nematode of sugar cane (Salawu, 1988). The powder of locust bean oil can also serve as a good biopesticide for the control of dried fish beetles (Odeyemi *et al.*, 2000). A wide variety of plants or plant products are known to be potentially toxic because they contain allelochemicals like glycosides, tannin, cyanides, alkaloids, phytic acid, hemagglutinins and saponins upon hydrolysis (Aletor, 1995). Harmful substances (toxicants) present in African locust bean pods are saponins, tannins, alkaloids and cardiac glycosides (Odeyemi *et al.*, 2000). These might have been released into the effluent during boiling or fermentation process and possibly through both processes. These toxic materials present in the African locust beans pods might have caused the death of the weevils when they are exposed to these effluents though mechanism of action and the exact active substance will be needed to be determined. The presence of nitrate and sulphate in effluent which can form acidic products could cause shortness of breath and might have resulted also in the death of the weevils (Odeyemi *et al.*, 2000). Phosphate is also present and is the basis of many insecticides (Jurewicz and Hanke, 2008). This is also in accordance with Alabi *et al.* (2005). They noted that a large percentage of mineral constituent may reside in the hull of locust beans seed and leached out during processing and that raw *Parkia biglobosa* contains some heavy elements like copper, lead and cobalt which are natural components of the earth crust and are toxic at low concentrations. The toxicity of locust bean effluent had also been reported by Anyanwu *et al.*, (2013) that exposed *Clarias gariepinus* juvenile to locust bean effluent and noted the adverse effect on their gills and livers. It resulted to rupture and eruption of their gills, leading to reduction in the respiratory surface area of the filament as well as impairment of oxygen-carbon (IV) oxide exchange.

### Conclusion

Fermented African Locust bean effluent as wastewater can be useful as biopesticide. This could be due to the production of toxin produced during processing and fermentation. Further research will be needed to determine which toxic fraction is responsible for the biopesticidal effect and the

fermentative condition needed for its release and also the boiling time and temperature required for the maximum release. The use of Fermented Africa locust bean effluent as biopesticide would be cheaper, less hazardous to human health and easily accessed by locals to preserve cowpea produce.

The lethal concentration i.e. concentration which to kill the insects would need to be determined.

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