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Research Paper

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Microbial Inhibitory Potency of Oven-Dried Mechanically Extracted Neem Seed Oil for Ecofriendly Pest Control in Food System

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Abstract—This research evaluated the anti-microbial inhibitory potency of oven-dried neem seed oil expressed mechanically for use as agricultural pest controller. The neem seed was evaluated for its moisture content and eco system friendliness. The moisture of the seed when evaluated is 9.07% which shows that high level bio-active compounds will be extracted when the oil is expressed. The ash content value is 0.133%. This shows that the neem seed oil has a negligible negative impact on the environment. The value of the oil yield mechanically expressed from neem seed oil in the research is 38.9%. The neem oil was expressed from the neem seed mechanically using a hydraulic press. The oil obtained was formulated into four concentrations of 25%, 50%, 75% and 100%. Each concentration was tested on four fungi species and four bacteria species. The fungi evaluated were *A. flavus, A. niger, P. notaium* and *Rhizopus nigricans* while the bacteria evaluated included *Escherichia coli, B. subtilis, R. meliloti* and *Pseudomonas aeruginosa*. From the mean results, the mean growth for both fungi and bacteria reduced as concentration increased. It was observed that *A. flavus* was completely inhibited as the percentage inhibition was 100% at 100% concentration of the neem seed oil. *A. niger, P. notatium* and *Rhizopus nigricans* recorded 96.06% 90.35% and 90.50%, respectively for 100% concentration. On the aspect of bacteria, complete inhibition was recorded for *Bascillus subtilus* and *Pseudomonas aeruginosa* while *E. coli and R. meliloti* recorded 96.27% and 97.28%, respectively at 100% concentration.

Keywords—Neem extract, Mechanical extraction, Percentage yield, Ash content, Microbial activities, Pest control, Food system.

I. INTRODUCTION

Microbes such as bacteria and fungi are major causes of pre and post-harvest loses in cereal crops like maize, and rice. They also cause lots of plant diseases [1, 2]. Typically, *Aspergillus, Penicillium, Fusarium* and some xerophytic species are the fungi genera common to stored grains. These common fungi genera have the ability to produce toxins that causes food spoilage and hazardous to stored food produce [3]. While [4] identified *Aspergillus* and *Fusarium* as plant-pathogenic fungi, with several species causing diseases on maize, wheat, barley etc. [5] reported the different concentration of *Aspergillus* and *Fusarium* as mycotoxigenic species in stored grains. According to [6] *Aspergillus flavus, A. parasiticus, A. nomius, A. tamarii* and *A. bombysis* is a toxigenic strains of *Aspergillus* section Flavi group produced as aflatoxins from the secondary metabolism of polyketide with *Aspergillus*. *flavus* being the main source of aflatoxins. [6] further noted that *Aspergillus*. *flavus* is the most important mycotoxins in the world's food supplies, due to its ability to increases the frequency of mutations above the natural background level, cause cancer in humans and teratogenic properties. In World-wide occurrence, there are 18 different types of *Aspergillus flavus*, and aflatoxin with Aflatoxin B1 (AFB1) and B2 (AFB2) the most important of them [7].

Neem extract is composed of a complex mixture of molecules, including normal hydrocarbons, phenolic compounds, terpenoids, alkaloids, and glycosides [8]. These molecules act on various phases of an insect's life cycle, making it difficult for pests to resist the physiological effects of neem extract [9].

Azadirachtin is the main bio-active constituent of neem extract which has been proved to have anti-feedant and toxic effects on insects [10] by energizing specific inhibition cells in mouth part chemoreceptors and also detering the firing of sugar receptor cells which stimulates feeding thereby causing starvation and death of insect species [11]. The antifungal and bacteria activity of neem tree extract is due to tetranortriterpenoid isomer being the major constituent of azadirachtin. Azadirachtin is well known as a potent phytogenic insect growth inhibitor [12]. Azadirachtin, extracted from neem oil, is both cheap and readily available on the market [13]. [14] reported that synthetic insecticides reduce seed damage better than neem extracts. However, neem based bio-pesticides do not affect the germination of stored cereal and legume seeds when it is treated with it [15]. In fact, [16] observed that rice seedlings raised from seed treated with 2.5 or 2% of the cake obtained after the extraction of oil from neem seed grew better and had higher root, shoot growth indices and dry weights than

the seedling raised from untreated seed. [17] noted that ecological, toxicological aspects, as well as economic aspects are some of the factors that have stimulated the use of neem- based products for pest control in agriculture. This is so because neem-based products have low toxicity to non-target organisms, the environment and human beings; small amounts of the product can provide effective pest control. Therefore, neem seed oil is very promising for the control of many pests [18, 19].

Oil extraction involves bringing out oil from agricultural seeds that contains oil [20]. These oil bearing agricultural seeds include conophor nut [21], olive [22], jojoba [23], and groundnut [24], and peanut kernel oil [25], soybean [26], sunflower [27], olive [22, 28], fish oil [29] and neem seed [30]. There are different extraction methods deployed to expel oil from seeds. These include the traditional method, supercritical fluid extraction method, mechanical method, solvent extraction process (contact equilibrium process), enzymatic extraction process or a combination of mechanical and solvent extraction processes [30, 31].

Mechanical extraction process is the application of pressure (using hydraulic or screw press) to force oil out of the oil bearing material. Mechanical extraction process is a more suitable method for both small and large (commercial) capacity operations; this may be due to the fact that it is economical compared with the other extraction methods. Mechanical extraction is the common method used to extract the neem oil from the seed [21, 32], since this method is effective for seeds containing 30-70% oil [33]. Usually the quality and quantity of the oil obtained by mechanical extraction process are affected by various operating conditions such as pretreatment of the neem seeds, extraction pressure, storage condition, moisture conditioning and size reduction [30, 34, 35, 36]. Therefore, the intention of this study is to investigate whether mechanical expression of neem oil at different particle sizes affects its microbial efficacy.

II. RELATED WORK

[37] evaluated the antimicrobial inhibitory property of different concentrations of extract from neem leaf. Using agar-well diffusion method, 13 microbial pathogens strains of animal origin were tested and the different concentrations were 10%, 50%, 75%, 90% and 100% while the tested microbial pathogen include *Citrobacter, Klebsiella, S. bodyi, S. sonnei, S. flexeneri, E. coli* O157, *E. coli* O78, *E. coli O26* and *Salmonella typhimurium* as well as Gram positive bacteria; *S. aureus* and MRSA as along with mycotic isolates; *C. albicans* and *Asp. flavus. The* results obtained from the research revealed that the neem extract has great inhibitory activities at lower concentrations of 10 and 50% than at 75, 90 and 100%. The result furthered showed bactericidal activities against Gram negative bacteria but did not against Gram positive bacteria for diluted neem extract. For 10% and 50% concentrations, *Citrobacter* recorded 14 and 12 mm, *Klebsiella* 19 and 18 mm, *S. bodyi* 18 and 17 mm, 18 and 15 mm against *S. sonnei,* 13 and 12mm against *S. flexeneri,* 14 and 12 mm against *E. coli* O157, 17 and 15 mm against *E. coli* O78, 14 and 13 mm against *E. coli* O26 and16 and 14 mm against *Salmonella*, respectively.

[38] investigated the Antibacterial activity of different concentration of neem (*Azadirachta indica*) seed oil extract against the following organisms *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* using microbial growth inhibition zone. The concentrations include 33.3%, 50%, 100% and Ampicillin 100% control. From the results, the rate of inhibition increased with increase in concentration for all tested organism with *Salmonella typhi* having the highest inhibition zones. Closing following *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* while *Escherichia coli* had the least zone of inhibition.

[39] conducted a research to evaluate the effect of aqueous, ethanolic and ethyl acetate extracts from neem leaves on growth of some human pathogens (*Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Candida albicans* and *Microsporum gypseum*) *in vitro* at different concentration. The different concentrations used in the evaluation are 5%, 10%, 15% and 20%. The growth inhibition of the test pathogens and gradually increased with concentration with the 20% ethyl acetate extract giving the strongest inhibition.

[40] evaluated the antibacterial activities of extracts from different parts (seeds, leaves, bark) of neem plant (*Azadirachta Indica)* against *Staphylococcus aurous*, *Staphylococcus epidermises*, *Acinetobacter baumanni*, *Psedommonas aeruginosa*, *and Escherishia coli* using Ager-well diffusion method. The research demonstrated anti-bacterial activities against all microorganisms tested for all parts of Neem parts. The results showed that neem seeds extract have significant effects for all tested bacteria, the maximum inhibition zone by seeds cold aqueous and cold ethanolic extracts were 22 & 13 mm for *E. coli* and *S. epidermidis* respectively; while leaves extracts were given 15 and 13 mm inhibitions zones against *S. aureus* and *E. coli* respectively.

The ability of different concentration of neem seed oil to inhibit the growth of *Escherichia coli a*nd *Staphylococcus aureus* was evaluated by [41]**.** The result showed varied inhibition tendencies of the different concentration of the extract which ranged from 20% to 100%. The level of inhibition was reduced when the concentration was between 20% to 40% and increased as concentration increased from 60 to 100mg/mmol. The result also showed that *E. coli* had more progressive inhibition zone as against *S. aureus.* **[41] also conducted a susceptibility test which showed** *Escherichia coli a*nd *Staphylococcus aureus* were resistant to the extract concentration from 0 to 40mg/mmol but become increasingly affected by the neem seed extract when the concentration reached 60% and above. They observed that affect both gram-positive and gram-negative bacteria.

In 2019, the rate of inhibition bacteria by Neem seed oil, Black seed oil and Mustard seed oil was investigated by [42]. The neem seed oil antibacterial inhibitory ability was determined on the selected organisms (*Staphylococcus aureus, Klebsiella pneumoniae, Salmonella Typhi* and *Pseudomonas Aeruginosa*) using in-vitro well diffusion method (Kirby and Bauer,1999).

Neem seed extract was able to hider the growth of the bacteria to more than 99% inhibition rate against the selected bacterial strains. The extracts of Black seed and Mustard seed also showed 99% ability to inhibit the growth rate of the selected bacteria strains except *Pseudomonas aeruginosa.* While the extract from the Black seed oil did not show any inhibitory potency, the extract from Mustard oil showed the little antibacterial activity against *Pseudomonas aeruginosa*. Of the oils tested, Neem seed oil showed that it was an effective antibacterial agent. In the same vain, the antibacterial ability of neem against *S. aureus,* cefepime, *S. typhi*, *E. coli* and *P. aeruginosa* were a subject of research by [43]. The authors used 'broth dilution method' to identify microbs while 'Agar disc diffusion method was used to evaluate bacterial susceptibility.' The result from the research showed an average diameter value of 19 mm against *S. aureus* for zone of inhibition. It further showed 30mm when the neem oil was tested against cefepime. *S. typhi, E. coli* and *P. aeruginosa.* exhibited. [44] Studied the anti-bacterial activity of fixed neem oil on Gram-negative bacteria, *E. coli* and Gram-positive bacteria, *S. aureus.* At 100mg/l concentration, the maximum inhibition zone for *E. coli* was 11.7mm after 2days (48hours). At 5, 20, 50, and 80 mg/mL concentrations, the values of inhibition zone were 8.7, 9.7, 10.3, and 11.3 mm respectively nurturing them for 48 h. While for *S. aureus*, the values were 8.7, 9.7, 10.7, 11 and 13mm of inhibition zone for 5, 20,50 and 80 and 100mg/mL respectively after a period of 48h.

III. METHODOLOGY

A. Preparation and Extraction of Neem Seed Oil

25kg of matured neem seeds were sourced from Unwana in Afikpo North Local Government Area of Ebonyi State, Nigeria and processed in the Department of Food Technology Akanu Ibiam Federal Polytechnic Unwana Afikpo North Local Government Area of Ebonyi State, Nigeria. Sourced neem seeds were cleaned and oven dried at a temperature of 65℃ for 16hours to allow for easy removal of the kernels [31, 45, 46]. The dried endocarps were then cracked to obtain the seed kernels; after decortication, the hulls of the seeds and other dirt were removed by winnowing. The moisture content and ash content of the seed was then calculated. [47]. The neem powder was then streamed over boiling water for about 20 minutes to allow the formation of the dough from which oil can be readily extracted. The oil was extracted from the dough with the aid of a hydraulic press and the percentage oil yield calculated.

B. Quality characterization

a. Moisture content of Neem seed

The percentage moisture in the kernel was calculated using the equation as described by [18]:

$$MC.(\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Where:
W_1 = Original weight of the sample before drying;
W2 = Weight of the sample after drying.

b. Ash content of Neem seed

Ash content of the organic sample is determined by the method described by **[**48**]** and calculated as:

$$Ash\ Content\ (\%) = \frac{W_3}{W_2} \times 100 \quad (2)$$

Where W_2= Ash(g); W_3= Sun dried specimen (g)
Organic matter (%) = 100-Ash content (%) (3)

c. Determination of percentage (%) yield

The percentage (%) yield was calculated using the equation [49, 50]

$$\%\text{yield of oil} = \frac{\text{Weight of oil}}{\text{Weight of sample (Neem seed)}} \text{ x } 100 \quad (4)$$

C. Determination of Effectiveness of Neem Seed Oil on the Test Organisms

Four (25%, 50%, 75% and 100%) concentrations of the neem seed oil were applied against the test organisms using the pour plate and the cork-boring methods described by [1]. The control was set up with tap water in place of the test oil.

a. Preparation of dilution of A. indica oil:

The produced *A. indica* oil was tested for its antimicrobial activity against four selected fungi and bacteria by preparing four concentrations of *A. indica* oil and one control (tap water) by dispensing the desired amount of oil in culture tubes containing desired amount of acetone under aseptic conditions.

b. Media Preparation and Culture of Fungi

[2] method of media preparation and culture was used with little modification. Potato Dextrose Agar (PDA) was combined with Chloramphenicol to isolate and enumerate fungi strains. The Plates for the inoculations were incubated at room temperature for 6 days. In accordance with [51 and for proper identification of the microbs, the microscopic and macroscopic features of the hypha mass, morphology of cells and spores, nature of the fruiting bodies, among other criteria were used.

c. Tests for Sterility of Extract

The sterility of the extracted oil was established by inoculating 1ml of the oil obtained by 9 ml of agar medium. The mixtures mixed in plates and incubated. The incubation did not show any growth on the mixture thereby confirming the extract, the environment and the apparatus used are sterile.

d. Determination of Percentage Inhibition

The control was measured at regular intervals of 24 hours for 5 days for its radial growth. The mean of the diameter obtained from the measurement was calculated and labelled X. The radial growth of each of the tested microbs was also measured and the mean calculated. It was labelled Y. The percentage inhibition of each organism by each concentration of the test oil was calculated using the formula:

$$\%\text{inhibition} = \frac{\text{growth in control} - \text{growth in treatment}}{\text{growth in control}} \times 100$$

D. Bacterial strains

Pure cultures of four bacterial species viz., *E. coli, B. subtilus, R. meliloti and P. aeroginosa* were obtained from Food Technology Department Akanu Ibiam Federal Polytechnic Unwana Afikpo North Local Government Area of Ebonyi State, Nigeria and stored on nutrient agar slants at 4º C for further study.

a. Inoculum preparation

Colony of each of the four bacteria were breed from pre-prepared nutrient agar culture and added to 5ml nutrient broth. It was nurtured at 37°C for 6 hours.

b. Preparation of disc containing different concentrations on neem oil:

The anti-growth ability of the neem extract was evaluated using filter paper (Whatman No.1) disc diffusion method. The Whatman filter paper discs of diameter 5mm were prepared with the help of a punching machine. The prepared discs were dipped in each concentration of neem oil, and control.

c. Preparation of seeded agar plates:

For a better bacterial suspension, 5ml of distilled water was added to inoculation tube and thoroughly shaken thoroughly. To four flasks containing 200ml of NAM each, 0.2ml suspensions of *Escherichia coli*, *Bascillus subtilus, R. meliloti* and *Pseudomonas Aeruginosa* were separately added in each flask. The mixture was maintained at 45° C to 50° C.

d. Transfer of seeded nutrient agar in sterilized Petri plates:

20ml of the inoculated medium was pipetted into six Petri plates for each of the bacterium. The Discs with the different oil concentrations were placed on the surface of NAM towards the periphery of the Petri plates along with the control disc in centre. The discs were placed in such a way that none of the appearing inhibition zone come together. The Petri plates were left open for 40-45 minutes uncontaminated. This is to allow the organic solvents to evaporate the organic solvents. The plates were inoculated at 37° C for 24 hours and inhibition zone measured in millimeter. The activity of respective solvents was subtracted from the total zone of inhibition. Growth inhibition (I) of bacteria was calculated by using the formula:

$$I = T - C$$

Where T = Diameter of total inhibition zone in treatment.
C = Diameter of inhibition zone in control.

IV. RESULTS AND DISCUSSION

Table 1: Quality Characterization of Extracted Neem Seed

S/N	Properties	units	Values
1.	Yield	%	38.9
2.	Moisture content (%)	%	9.07
3.	Ash Content (%)	%	0.133

FUNGI

Table 2: Effect 25% Neem Seed Oil conc. on the radial Growth of Some Fungi Species

Fungi	Days					Mean
	1	2	3	4	5	(mm)
A. flavus	1.87	2.01	2.65	3.08	3.23	2.57
A. niger	2.25	2.93	3.08	3.45	4.19	3.18
P. notatium	2.48	3.89	4.32	5.63	6.50	4.56
R. nigricans	3.67	4.00	4.16	4.98	5.77	4.52

Table 3: Effect 50% Neem Seed Oil conc. on the radial Growth of Some Fungi Species

Fungi	Days					Mean
	1	2	3	4	5	(mm)
A. flavus	1.00	1.05	1.10	1.25	1.45	1.17
A. niger	1.20	1.77	1.96	2.34	2.67	1.99
P. notatium	1.67	2.00	2.21	2.47	2.55	2.18
R. nigricans	2.03	2.11	2.40	2.57	2.63	2.35

Table 4: Effect 75% Neem Seed Oil conc. on the radial Growth of Some Fungi Species

Fungi	Days					Mean
	1	2	3	4	5	(mm)
A. flavus	0.40	0.73	0.96	1.03	1.10	0.81
A. niger	0.50	0.65	1.02	1.07	1.07	0.86
P. notatium	1.05	1.60	1.70	1.80	1.85	1.6 0
R. nigricans	1.66	1.85	1.93	2.01	2.05	1.90

Table 5: Effect 100% Neem Seed Oil conc. on the radial Growth of Some Fungi Species

Fungi	Days					Mean
	1	2	3	4	5	(mm)
A. flavus	0.0 0	0.00	0.00	0.01	0.00	0.00
A. niger	0.31.	0.33	0.37	0.38	0.41	0.36
P. notatium	1.01	1.01	1.02	1.02	1.02	1.02
R. nigricans	1.05	1.16	1.20	1.23	1.25	1.12

Table 6: Effect Tap Water Control Set up* on the radial Growth of Some Fungi Species.

Fungi	Days					Mean
	1	2	3	4	5	(mm)
A. flavus	4.50	5.80	7.77	10.19	12.01	8.05
A. niger	4.80	7.10	10.97	14.34	17.33	10.91
P. notatium	7.38	9.22	10.56	11.69	14.00	10.57
R. nigricans	6.15	9.43	12.56	13.97	16.85	11.79

Table 7: %Percentage Inhibition of Fungi species treated with 25% Neem Oil

Fungi Species	Mean Radial Growth			
	Neem Oil	Control	Difference	%Percentage Inhibition
A. flavus	2.57	8.05	5.48	68.07
A. niger	3.18	9.13	5.95	65.17
P. notatium	4.56	10.57	6.01	56.86
R. nigricans	4.52	11.79	7.27	61.66

Table 8: %Percentage Inhibition of Fungi species treated with 50% Neem Oil

Fungi Species			Mean Radial Growth	
	Neem Oil	Control	Difference	%Percentage Inhibition
A. flavus	1.17	8.05	6.88	85.47
A. niger	1.99	9.13	7.14	78.20
P. notatium	2.18	10.57	8.39	79.38
R. nigricans	2.35	11.79	9.44	80.07

Table 9: %Percentage Inhibition of Fungi species treated with 75% Neem Oil

Fungi Species	Mean Radial Growth			
	Neem Oil	Control	Difference	%Percentage Inhibition
A. flavus	0.81	8.05	7.24	89.94
A. niger	0.86	10.91	10.05	92.12
P. notatium	1.6 0	10.57	8.97	84.86
R. nigricans	1.90	11.79	9.89	83.88

Table 10: %Percentage Inhibition of Fungi species treated with 100% Neem Oil

Fungi Species			Mean Radial Growth	
	Neem Oil	Control	Difference	%Percentage Inhibition
A. flavus	0.00	8.05	8.05	100 .00
A. niger	0.36	9.13	8.77	96.06
P. notatium	1.02	10.57	9.55	90.35
R. nigricans	1.12	11.79	10.67	90.50

BACTERIA

Table 11: Effect 25% Neem Seed Oil Conc. on the Radial Growth of some Bacterial strains.

Bacterial	Days					Mean (mm)
	1	2	3	4	5	
E. coli	6.05	7.30	10.70	13.09	17.7	10.97
B. subtilus	5.98	7.68	9.87	12.30	15.88	10.34
R. meliloti	7.45	10.61	13.23	16.06	21.00	13.67
P. aerugenosa	4.90	6.70	9.03	11.50	14.75	9.38

Table 12: Effect 50% Neem Seed Oil Conc. on the Radial Growth of some Bacterial strains.

Bacterial	Days					Mean (mm)
	1	2	3	4	5	
E. coli	2.98	3.23	3.68	4.01	4.67	3.71
B. subtilus	2.61	3.00	3.79	4.14	4.78	3.66
R. meliloti	3.11	3.57	3.90	4.35	5.00	3.99
P. aerugenosa	3.12	3.76	4.51	4.90	5.34	4.33

Table 13: Effect 75% Neem Seed Oil Conc. on the Radial Growth of some Bacterial strains.

Bacterial	Days					Mean (mm)
	1	2	3	4	5	
E. coli	1.34	1.42	1.70	2.08	2.23	1.75
B. subtilus	1.43	1.59	1.88	2.15	2.35	1.88
R. meliloti	2.01	2.19	2.67	3.03	3.17	2.61
P aerugenosa	1.30	1.79	2.01	2.12	2.23	1.89

Table 14: Effect 100% Neem Seed Oil Conc. on the Radial Growth of some Bacterial strains.

Bacterial	Days					Mean (mm)
	1	2	3	4	5	
E. coli	0.56	0.65	0.73	0.84	0.84	0.72
B. subtilus	0.00	0.00	0.00	0.00	0.00	0.00
R. meliloti	0.34	0.46	0.64	0.77	0.90	0.62
P. aerugenosa	0.00	0.00	0.00	0.00	0.00	0.00

Table 15: Radial growth of some Bacterial strains treatment with Tap Water control set up*

Bacterial	Days					Mean (mm)
	1	2	3	4	5	
E. coli	14.02	17.35	19.71	21.56	23.87	19.30
B. subtilus	13.79	18.65	21.00	23.45	25.94	20.57
R. meliloti	15.25	19.30	23.12	26.66	29.80	22.83
P. aerugenosa	11.00	14.57	17.01	20.61	22.00	17.04

Table 16: %Percentage Inhibition of Bacterial Strains treated with 25% Neem Oil

Bacterial	Mean Radial Growth			
	Neem Oil	Control	Difference	%Percentage Inhibition
E. coli	10.97	19.30	8.33	43.16
B. subtilus	10.34	20.57	10.23	49.73
R. meliloti	13.67	22.83	9.16	40.12
P. aerugenosa	9.38	17.04	7.66	44.95

Table 17: %Percentage Inhibition of Bacterial Strains treated with 50% Neem Oil

Bacterial	Mean Radial Growth			
	Neem Oil	Control	Difference	%Percentage Inhibition
E. coli	3.71	19.30	15.59	80.78
B. subtilus	3.66	20.57	16.91	82.21
R. meliloti	3.99	22.83	18.84	82.52
P. aerugenosa	4.33	17.04	12.71	74.59

Table 18: %Percentage Inhibition of Bacterial Strains treated with 75% Neem Oil

Bacterial	Mean Radial Growth			
	Neem Oil	Control	Difference	%Percentage Inhibition
E. coli	1.75	19.30	17.55	90.93
B. subtilus	1.88	20.57	18.69	90.86
R. meliloti	2.61	22.83	20.21	88.52
P. aerugenosa	1.89	17.04	15.15	88.91

Table 19: %Percentage Inhibition of Bacterial Strains treated with 100% Neem Oil

Bacterial	Mean Radial Growth			
	Neem Oil	Control	Difference	%Percentage Inhibition
E. coli	0.72	19.30	18.58	96.27
B. subtilus	0.00	20.57	20.57	100
R. meliloti	0.62	22.83	22.21	97.28
P. aerugenosa	0.00	17.04	17.04	100

Discussion

A. Quality Charaterization

Table 1 shows the quality characteristic of the neem seed and the extracted oil. The parameters that were evaluated include, moisture content of the seed, the ash content as well as the percentage (%) yield of the oil.

a. *Moisture content of Neem seeds*

The ability of a solvent to diffuse or transfer to the surface of a seed or food material is a function of the moisture content of the mater this is because moisture blocks the diffusivity of solvent to the seed surface thereby stopping the extraction of the bio-active compounds present in the seed as oil. In other words, moisture content of the seed indicates the extraction kinetic of the oil in the seed [18]. Thus moisture content is inversely proportional to the yield content of extractable bio-active compounds [31]. [52] reported that the standard range of moisture content needed to obtain neem oil with high bio-active ingredients is 9.04-9.10. From the result obtained from this research, the moisture content of the oil produced is recorded at 9.07%. which corresponds with the range given for standard moisture content. This result is in agreement with earlier work reported by [31, 49] and lower than that obtained by [20, 48, 53]. From the result and discussion, the oil extracted mechanically from an oven-dried neem seed in this research has a high level of biologically active compounds.

b. *Ash contents of Neem seeds*

The ash content of a material represents the non-volatile and non-decomposable compounds which are harmful to the environment. It therefore implies that ash content shows the friendliness of a substance to the eco system. Hence, the ash content of neem seed simply shows the eco-friendliness of neem seed represented by the nonorganic matter or compounds of neem seed [18]. The ash content which is mostly metallic oxides cause different kinds of health issues on different floral and faunas. The higher the quantity of nonorganic material the more the substance is not ecofriendly. Therefore, the value of the ash content should be as small as possible. From table 1, the ash content of the neem seed is 0.133% of total weight of the matter which therefore gives the organic content of the neem seed to be 99.867%. This shows that the extracted neem seed oil is biodegradable and environmental sustainable and can be used comfortably on the environment with little or no side effect to the environment and its living organization. This finding is lower than the 10% ash content reported by [54], and the 12% reported by [55] but agrees relatively with the 0.6% reported by [56], 0.143 obtained by [18], 0.12 by [57] and the 0.183% of [50].

c. *Percentage Oil Yield*

The efficiency of the extraction method is evident in the quantity of oil extracted from the yield. From literature, the quantity of extractable oil from the dried seed mass can be as high as 25-49% of the dried mass [58, 59, 60]. From the result obtained, the extraction of the oil from Neem seed using mechanical extraction method was viable compared to result of similar methods as a yield percentage of 38.9% was recorded. This result was above the 21.32% result obtained by [57]. [31] reported a range of results for different moisture content of mechanically extracted neem oil. These range include, 22.37% (at 6.3% moisture content), 24.86% (at 8.1% moisture content), 21.21% (13.2% moisture content) and 15.62% (at 16.6% moisture content) while [30] reported a yield of 32% which similar to the one obtained in this research. The increase in the oil yield in this research compared to other similar extraction method could be as a result of the pre-treatment (steaming) given to the crushed seed before extraction. However, when mechanically expressed, the yield is less than when express using solvent extraction method as shown from the result obtained by It was however lower that the results obtained by [18, 47, 61, 62, 63] that used hexane and ethanol as the solvent for extraction and reported 44.6, 43.71%, 44%, 43.48% and 44.29% respectively.

B. Fungi Species

[64] observed that because of the compositional complexity of neem tree, neem extract and neem-based products can act as anti-feedants, growth regulators, sterilants, anti-oviposition agents, and repellents. Tables 2, 3, 4, 5 and 6 show the growth rate in millimeter of the different fungi species when treated with different concentrations of the oven-dried mechanically extracted neem seed oil. From the results obtained, the growth rate decreased as concentration and days of inoculation increased as can be seen from their radial mean growth. From the mean results, *A. flavus* had the most reduced growth*;* 2.57mm at 25% neem oil concentration to 0.00mm at 100% concentration, followed by *A. niger* whose growth was reduced from 3.18mm at 25% to 0.36mm at 100% of Neem oil. *P. notatium* and *Rhizopus nigricans* were reduced from4.56mm and 4.52mm at 25% to 1.02mm and 1.12mm at 100% Neem oil concentration respectively. This shows that the Neem oil has the highest inhibitory effect on the *A. flavus* and the least on *Rhizopus nigricans*. When compared with the research control, the Neem oil of all concentrations was able to effectively inhibit the growth of the fungi. The results obtained for *A. flavus and niger* were smaller than that reported by [58] which put the growth of the *A. flavus and niger* at 11mm and 10mm respectively with 60% concentration. However, [2] reported a mean growth of 2.84mm for *Aspergillus spp* and 11.00mm for *Rhizopus nigricans* while [59] reported a spore count of 12, 9 and 3 for 0.1 0.2 and 0.5 concentration of Neem oil. Also, [13] evaluated the reproduction ability of micro-organisms in

Commercial Soil and Bangpakong soil when mixed with azadirachtin. From the research, it was found that both samples had significantly lesser number of microorganisms than the control plates when mixed with azadirachtin at any concentration.

Tables 7, 8, 9 and 10 show the percentage of inhibition of the various neem oil concentrations when compared with the control. It was observed that for *A. flavus*, the percentage inhibition was 68.07%, 85.47%, 89.94% and 100%; *A. niger* recorded 65.17%, 78.20%, 92.12% and 96.06%; *P. notatium* has 56.86%, 79.38%, 84.86% and 90.35% while *Rhizopus nigricans* 61.66%, 80.07%, 83.88% and 90.50% for 25%, 50%, 75% and 100% concentrations, respectively. Thus it can be inferred that neem oil is a good pest controller. [60] reported a percentage inhibition of 35.22%, 49.55% and 100% on *A. niger* for 5%, 10% and 15% concentration. the result, *A. flavus* inhibited completely at 100% neem oil concentration followed by *A. niger*. [59] reported 52%, 64% and 88% percentage inhibition on *A. flavus* when 0.1, 0.2 and 0.5 concentrations were used. [65 F. Rashid, f. Naaz, M. Z. Abdin, S. Zafar, S. Javad] recorded complete inhibition of *Aspergillus spp* at 50µml^{-1}. [6] evaluated the application of aqueous extract from neem leaves in cotton balls infected with *Aspergillus flavus*. The research observed that the neem leave extract was able to inhibit up to 98% the AFB1 production, without reducing the mycelial growth. The addition of the aqueous neem leaf extract in submerged cultures of *Aspergillus parasiticus* over 5.0% concentrations caused inhibition of over 90% in AFB1 production, but did not affect mycelial growth. [6] suggested that the inhibitory components in these extracts are non-volatile. From the results obtained, the best concentration for the treatment of the understudied fungi was 100% as it produced the best results from the research.

C. Bacterial Strains

The growth of the bacterial strains evaluated in this work was inhibited by the Neem seed oil extract. The mean growth rate of the bacterial strains decreased as the concentration increased on all bacterial strains with *B. subtilus* and *Pseudomonas aerugenosa* experiencing complete inhibition when the concentration was at 100% as shown in Table 11, 12, 13, 14 and 15.

When compared with the control in Table 15, the result from table 16, 17, 18 and 19 show that the %percentage of inhibition of the bacterial increased as the concentration of neem extract increased with the highest percentage inhibition occurring at 100%. At this concentration, *E. coli* has 96.27% inhibition, *B. subtilus* and *P. aerugenosa* has 100% inhibition while *R. meliloti* has 97.28% inhibition. [66] observed that the seed and fruit extracts of neem tree showed antibacterial activity only at higher concentrations. The above statement is true for the results obtained as the concentration of the seed extract increased. At 25% concentration, the research recorded 43.16%, 49.73%, 40.12%, and 44.95% for *Escherichia coli, B. subtilus, R. meliloti* and *Pseudomonas Aeruginosa* respectively while at 50% concentration, the % percentage inhibition of the oil almost doubled for all the bacterial strains evaluated as can be seen in Table 17. Tables 18 shows that the %percentage inhibition of *Escherichia coli, B. subtilus, R. meliloti* and *Pseudomonas Aeruginosa* by the oil is 90.93%, 90.86% 88.52% and 88.91% respectively for 75% concentration. At 100% concentration, complete inhibition was recorded for *Bascillus subtilus* and *Pseudomonas Aeruginosa* whle *E. coli and R. meliloti recorded* 96.27% and 97.28% respectively. The results obtained in this result tend to agree with earlier result reported by [43] for *Pseudomonas Aeruginosa* and *E. coli* were they recorded 92% susceptibility for both strains at 65% concentrations. [67] reported 91.51% inhibition for *Escherichia coli* and 88.90% for *Pseudomonas fluorescens* respectively. [42] reported 100% inhibition for *Pseudomonas Aeruginosa* when the concentration of neem seed oil was 100%. The susceptibility of bacteria to the seed extracts at higher concentrations may be due to the strains of the isolates being bacteriostatic at lower concentrations.

V. Conclusion and Future Scope

The inhibitory tendency of four concentrations of oven-dried mechanically extracted neem seed oil on agricultural pests was tested on four fungi and bacterial strains for eco-friendly pest control in food system. The yield of the oil extracted using the chosen method of extraction, was high compared results obtained by other researchers that used the same method. The oil which were in four different concentrations namely 25%, 50%, 75% and 100% showed inhibitory ability. This showed that the method of extraction did not in any way reduce the efficacy of the extracted neem seed oil. The plant extract successfully reduced the growth of all the micro-organisms tested. For both fungi species and bacterial strains, complete inhibition was recorded for *Aspergillus flavus, B. subtilus* and *Pseudomonas Aeruginosa* while the rest of the microbs recorded over 90% inhibition after 5 days of treatment when 100% concentration of the plant extract. It can therefore, be concluded that mechanically extracted neem seed oil has high efficacy when used in an agricultural pest control and this would enhance maximal food production thereby ensuring food security. It is recommended that further work should be done to identify the specific bio-active compound(s) that causes this inhibitory effect so that it can be purified and standardize as a preservative against microbial contaminant of food.

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