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Research

COMPARATIVE BIO-ACTIVITY OF SOME EDIBLE SPICE POWDERS IN THE CONTROL OF THE MAIZE STORAGE WEEVIL (*Sitophilus zeamais Motsch)*

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Abstract

Five edible plant product powders (EPPP), West African black pepper (WABP) (Piper guineense Schum and Thonn.), clove (Syzgium aromaticum (L.) Merril & Percy), Ethiopian pepper (Xylopia aethiopica (Dunn) A. Rich), Alligator pepper (Aframomum melegueta Schum) and ginger (Zingiber officinale Rosc.), were studied for their acute toxicity and effectiveness in repelling, acute toxicity, oviposition and progeny emergence suppression of Sitophilus zeamais (Motsch) activities at the rate of 0.5, 1.0, 1.5 and 2.0 g/10g maize seeds. The experiment was carried out as treatments before infestation (TBI) and treatment after infestation (TAI), in the laboratory under ambient temperature and relative humidity. All the dosage rates tested evoked repellency class of III and IV in all the (EPPP) treatments, while the control evoked class II. The results of the acute toxicity showed that all the EPPP can cause mortality of S. zeamais albeit at low dosage rates. Thus, the EPPP can be ranked in order of toxicity as WABP > Clove > Ginger > Alligator pepper > Ethiopian pepper. All the plant product powders significantly ($P \leq 0.05$) reduced oviposition by S. zeamais when tested as treatment before infestation, compared with control treatments. P. guineense and S. aromaticum at all dosage rates completely inhibited progeny emergence. The activities of A. melegueta, X. aethiopica and Z. officinale were dose dependant in reducing progeny emergence ($P \leq 0.05$). In the case of treatment after infestation progeny, emergence was least in treatments with WABP, clove and Ethiopian pepper. There were however, significant differences ($P \leq 0.05$) between the EPPP and the control treatment at all dosage rates. The potential effectiveness of all the EPPP implies that stored maize could be adequately protected against S. zeamais thus reducing infestation.

Key words: West African black pepper, clove, ginger, Ethiopian pepper, Alligator pepper, repellency, toxicity, treatment before infestation, treatment after infestation.

Introduction

Maize, *Zea mays* (L.) is a large, annual monoecious grain of the small tribe Maydeae, which belongs to the family Gramineae. Maize was the principal food of the ancient civilizations of the Western Hemisphere: the Incas of Peru, the Mayas of Central America, the Aztes of Mexico and some parts of eastern North America (Ruskin, 1988). According to Ruskin (1988), maize is grown on over 133 million hectares, from latitude 40^{o}S in Argentina to latitude 58^{o}N in Canada and the Soviet Union. Maize is planted in wider range of climatic conditions than rice and wheat due to its adaptability in warm temperate regions as well as the humid subtropical regions (Olukosi and Raphael, 1997).

Maize is the most important cereal crop in Africa, and one of the world's three most important cereal crops after rice and wheat (David, 1985; Asiedu, 1989). The crop is mainly grown for its grain, which is utilized for human consumption and forms about 50- 70% of the constituents of livestock feeds (David, 1985). It is relatively inexpensive and provides many families with the much needed carbohydrate. Maize provides in small amount proteins, fats, vitamins and minerals (Kling and Edmeades, 1997). It also provides raw ingredients for industrial materials and supports a worldwide business worth $40 million annually (Ruskin, 1988). Maize is grown by most households annually, either as a vegetable at the back of the house or on the farm.

Maize production has shifted from being a predominantly food crop to a major income earner for farmers in many countries in Africa. Maize production increased from 2.06 million tons in 1984 to 5.77 million tonnes in 1990 in Nigeria (Valentia, 1999). Kehinde (1997) reported that maize is now the second most important cereal crop in Nigeria. Maize production in the world in the last 5 years outranked paddy rice and wheat, with about 60% produced in the developed countries, particularly USA. China produced 27% of the world's maize and the rest is produced in Latin America, Africa and Southern Asia with the largest production being produced in the tropics and subtropics (Luc *et al*., 2005).

Excess grains after production season may be stored for use in subsequent times. Food grains are stored for various reasons (Jugenheimer, 1976; Ruskin, 1988; Asiedu, 1989; Valentia, 1999; Lale, 2001). During storage, maize grains could be infested by a catalogue of stored product pests. One of the most devastating is the maize weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera:Curculionidae) (Haines, 1991; Asawalam, 2006). The maize weevil is capable of developing on all cereal grains and cereal products (Walgenbach and Burkholder, 1986; Bekele, 2002). Pre-harvest losses attributed to maize weevil were about 8- 10% and this continues during post harvest storage. Losses of up to 30-50% were recorded for maize stored for upward of six months in Nigeria (Taylor, 1971); and Schulten (1989) also reported about 10% loss in stored cereals due to pest infestation in Nigeria. World annual losses due to post harvest losses as a result of pest infestation had been put between 25 and 40% (Hill and Waller, 1990). The infestation and developmental activities of the weevil often lead to severe powdering, considerable loss in weight, deterioration of quality, tainting of grains with excrements and exuviae, increase in grain humidity and subsequent rapid growth of microflora (Adedire, 2001; Lale, 2002). This may cause to cake with subsequent reduction in market values and organoleptic properties of the grains (Adedire, 2001; Lale, 2002; Oparaeke, 2004).

Often times, the control of this pest relies heavily on the use of synthetic insecticides. However, the negative environmental impacts, increasing cost of application, development of resistant strains (Schwab *et al*., 1995); worker's safety and erratic supply in developing countries due to foreign exchange constraints have necessitated research interest on the development of alternative insect pest control measures (Berger, 1994). Peasant farmers the world over often claimed successful use of materials of plant origin in insect pest control including ash, ground pepper, spices and powders of plant parts (Lajide *et al.,* 1998). The plant products used for this study have been used previously in the control of some stored products (Grainge and Ahmed, 1988; Lale, 1992). The objective of this study was to compare the biological efficacy of five different edible plant product powders (EPPP) in controlling the maize weevil, *Sitophilus zeamais* (Motsch.) infesting stored maize in Nigeria.

Materials and Methods

Preparation of Plant Materials. Dry seeds of *Syzgium aromaticum* (Clove), *Piper guineense* (West African Black pepper), *Aframomum melegueta* (Alligator pepper), *Xylopia aethiopica* (Ethiopian pepper) and dry rhizome of *Zingiber officinale* (ginger) were purchased from the market in Lafia (08^{o} 33'N and 08^{o} 32'E) north central, Nigeria. The seeds and rhizomes were separately ground into powder using pestle and mortar. The powders were then sieved using a fine pore sieve (2 mm) and the powders were separately placed in transparent polyethylene bags and stored in the laboratory under ambient laboratory (25 – 30 ^{O}C and 75 – 80% RH) until ready for use.

Insect Rearing and Maintenance. Adult maize weevils (*S. zeamais)* used for the study were obtained from an already infested maize grains in Lafia market, Nasarawa State, Nigeria in April, 2007. From this stock, new generations were reared in the laboratory on local variety of maize at room temperatures of 25-30^0 C and relative humidity of 75-80%. Freshly emerged adults of *S. zeamais* were then subsequently re-infested on the same variety of maize over five generations to acclimatize before they were used for the experiments.

Preparation of Maize Seeds. Four kilogrammes of clean and sorted maize seeds (Buhu Banza) used for the experiment was purchased from Musha village in Obi Local Government Area of Nasarawa State, Nigeria. The maize seeds were kept inside the deep freezer at -0^o C for three months in order to kill any insect pests present. The seeds were later air dried for three consecutive days to allow seeds to equilibrate.

Experiment 1: Toxicity and Repellency Tests of Edible Plant Product Powders:

Acute Toxicity Tests. Powders of *S. aromaticum, P. guineense, A. melegueta, X. aethiopica* and *Z. officinale* were each weighed at five dosage rates (0, 0.5, 1.0, 1.5 and 2.0 g) and admixed with 10 g of pristine maize grains in a 50 ml glass jar. Each dosage rate was replicated three times. Thereafter, 25 adults of 3-5 day old *S. zeamais* were introduced into the jars and the lids were replaced to cover the jars. The experiment was set up in a completely randomized design (CRD). The experiment was allowed to stay for 24 hours before mortality of the weevils were taken.

Repellency Tests. An adapted method of Mohan and Fields (2002) was used for the repellency test. The method involved the use of a wire meshed cup (perforated cone chamber (PCC) with an iron ring at the mouth to hold it firmly and a cut carton placed at the bottom of the cup to hold in place the EPPP. Five dosage rates of each EPPP (0, 0.5, 1.0, 1.5 and 2.0 g) were admixed with 10 g of pristine maize grains placed in the PCC. Each dosage rate was replicated three times, and 25 adult *S. zeamais* were introduced into the perforated cone chamber (PCC). Thereafter, the number of *S. zeamais* present in the PCC and the number that moved out were recorded after 30 minutes. The experiment was set up in a completely randomized design (CRD).

Experiment II. Bioassay Test of five Edible Plant Product Powders (EPPP) on the Biology of *S. zeamais*

Treatment Before Infestation (TBI) *of Edible Plant Product Powders* (EPPP). Powders of

S. aromaticum, P. guineense, A. melegueta, X. aethiopica and *Z. officinale* were each weighed at four dosage rates (0.5, 1.0, 1.5 and 2.0 g) and admixed with 10 g of pristine maize in a 50 ml bottles. Each dosage rate was replicated three times. Thereafter five (5) pairs of 3-5 day old adults *S. zeamais* were introduced into the bottles. The control batches were set along with the treatments without any admixture of EPPP. Adult female *S. zeamais* were allowed to mate and oviposited for 20 days, before both live and dead insects were all removed. The experiment was set up in a completely randomized block design. Thereafter, the treatments were allowed to stand in the laboratory until progeny adult emergence. Adult *S. zeamais* that emerged were removed and recorded daily for upwards of three months in each treatment and replicates.

Treatment After Infestation (TAI) *of Edible Plant Product Powders* (EPPP). In the case of treatment after infestation (TAI) the same number of insects was used. The pairs of insects were first of all allowed to mate and allowed to oviposit on pristine maize seeds (10 g) weighed into 50 ml bottles for a period of 20 days before both live and dead insects were removed. As reported in TBI each of the EPPP were weighed out and applied at the same dosage rates. Each treatment was shaken very well in order to allow proper mixing of EPPP with the grains. Subsequent procedures were identical with those followed in the treatment before infestation. The experiment was set up in a completely randomized block design.

Data Analysis. Data relating to Toxicity test was analyzed using a U.S.E.P.A Probit Analysis Programme Version 1.5. Data relating to repellency test were calculated according to the method of Sighamony *et al.* *(*1984). Repellency classification was done according to the method of McGovern *et al.* (1977). Data relating to treatment before infestation and treatment after infestation were subjected to one way analysis of variance (ANOVA) by using a GENSAT 2005 programme package. Means were compared using the Least Significant Difference (LSD) test at P = 0.005 (5%).

Results and Discussion

Table 1 represents the mean repellency values of five (5) edible plant product powders (EPPP) at different dose levels against *S. zeamais* in the stored maize grains. More number of adult *S. zeamais* was significantly repelled from Alligator pepper in the perforated cone chamber (PCC) than other EPPP. The repellency actions of the EPPP were dose dependant as can be seen in Table 1. All the EPPP were statistically, different from the control (Table 1). At the highest dosage rate of 2.0 g/10 g maize grain, all the EPPP repelled significantly ($p \leq 0.05$) higher number of adult *S. zeamais* when compared to the control except for that 0.5 g/10 g maize grain treated with alligator pepper. Thus, all the EPPP evoked repellency classes of III – IV while the control evoked class II (Table 2).

Table 1: Mean number of S*itophilus zeamais* adults repelled from repellent cone chamber treated with different five edible plant product powders (EPPP).

Dosage (g/10 g maize)	Source of edible plant product powders				
	Alligator pepper	Clove	Ethiopian pepper	Ginger	WABP*
0	25.60	29.41	29.93	29.60	28.30
0.5	62.20	47.49	55.25	51.50	44.40
1.0	71.00	64.59	65.75	62.20	58.80
1.5	71.90	68.12	70.65	68.30	67.00
2.0	73.60	76.10	74.78	76.10	74.80
Mean	60.90	57.14	59.27	57.50	54.60
SED	3.11	1.96	1.58	2.58	3.90
LSD	10.14	6.39	5.15	8.41	12.72

*WABP = West African black pepper.

Table 2: Classification of repellent action of five edible plant product powders (EPPP) against adults *Sitophilus zeamais* infesting stored maize grains.

	Alligator pepper		Clove		Ethiopian pepper		Ginger		WABP*	
Dosage (g/10 g maize)	Mean (%)	Rep. class	Mean (%)	Rep. class	Mean (%)	Rep. class	Mean (%)	Rep. class	Mean (%)	Rep. class
0	25.60	II	29.41	II	28.93	II	29.60	II	28.30	II
0.5	62.20	IV	47.49	III	55.25	III	51.50	III	44.40	III
1.0	71.00	IV	64.59	IV	65.75	IV	62.20	IV	58.80	III
1.5	71.90	IV	68.12	IV	70.65	IV	68.30	IV	67.00	IV
2.0	73.60	IV	76.10	IV	74.78	IV	76.10	IV	74.80	IV

*WABP = West African black pepper.
Repellency class =

Class I = 10-20%

Class II= 20.1 – 40%

Class III = 40.1 – 60%

Class IV = 60.1 – 80%

Class V = 80.1 – 100%

The use of plant product also has been reported to show some repellent efficacy against stored product pests Asawalam *et al.* (2006) reported that essential oils of *Ocimum suave* evoked different classes of repellency on *S. zeamais*. In another report Hassanali *et al*. (1990) demonstrated the repellent effect of essential oils of *Ocimum suave* leaves and the dried unopened flower buds of *Eugenia caryophyllata* against *S. zeamais*. The report of this study has shown that the EPPP were capable of repelling *S. zeamais* as much as the use of essential oils. It has been suggested that some plant products releases volatile oils when crushed (Lale, 1992). The volatile oils contained in the EPPP might be the source of repellent action in the EPPP used.

The irritant and foul smelling chemical exudates from some plant product have been reported to repel species of *Tribolium* (Asawalam, 2006). It has also been reported that *T. castaneum* adults were significantly repelled by neem seed extract, whereas *Rhyzopertha dominica* (F.) was less affected (Lale and Alaga, 2001). *Syzigium aromaticum* is highly pungent and contains eugenols, sesquiterpene and caryophiline. The oil extracts applied on filler paper showed class IV repellency against another Curculonid, *T. castaneum* (Dales, 1996; Asawalam, 2006). Lale and Alaga (2001) reported that *P. guineense* seed oil evoked a repellency class V in *T. castaneum* adults, in all the dosage rates tested in stored pear millet. Obeng-Ofori *et al.* (1997) reported that eugenol evoked a repellency class of 80 – 100% (V) in adults of *T. castaneum*. The strong repellent properties of *P. guineense, S. aromaticum, Z.officinale, X. aethiopica* and *A. melegueta* powders implies that stored maize may be adequately protected against population of *S. zeamais* thus reducing their infestation level. The repellent properties demonstrated against *S. zeamais* suggest that *P. guineense, S. aromaticum, Z. officinale, X aethiopica* and *A. melegueta* have broad spectrum of biological activities. They are known to contain different alkaloids, eugenol, cayophyline, paradol that possibly constitute their active ingredients (Lale, 1992; Lale and Alaga, 2001). Hence, they significantly protect maize against weevil attack.

The acute toxicity tests with the EPPP (Tables 3) showed that *P. guineense* with an LC_{50} of 0.427 g/10 g maize seed and fiducial limits of 0.101-0.649 g/10 g maize seed was the most toxic followed by *S. aromaticum* with an LC_{50} of 0.694 g/10 g maize seed and fiducial limits of 0.376 – 0.927 g/10 g maize seed. The LC_{50} for *Z. officinale* was 1.629 g/10 g maize seed and its corresponding fiducial limits were 1.120-3.740 g/10 g maize seed. *A. melegueta* with an LC_{50} of 3.038 g/10 g maize seed and fiducial limits of 1.732-10.128 g/10 g maize seed and the LC_{50} of *X. aethiopica* was 3.300 g/10 g maize seed with fiducial limits of 2.056 – 5.711 g/10 g maize seed. LC_{50} has been reported to be the concentration of an insecticide (or any pesticide) in acute dermal toxicity required to kill 50% of the test insects (animals) (Lale, 1996). In this report, the fiducial limit ranges for each of the EPPP were the lethal concentration ranges at which each of the EPPP can cause 50% mortality of the test insects. The order of ranking of the EPPP in terms of their toxicity was WABP > Clove > Ginger > Alligator pepper > Ethiopian pepper.

The five EPPP demonstrated significant toxic effects against *S. zeamais* adults. The results, particularly those of *P. guineense* and *S. aromaticun* are consistent with those of earlier workers (Lale, 1992). In the study of Lale (1992), the oil extract of *P. guineense* was 9 times as toxic as *Dennettia tripetala* and of a higher order of toxicity and when compared with *A. melegueta* it was 2.0 and 1.1 times as toxic as *P. guineense*, respectively. In this present study, the toxicity of *P. guineense* was 0.3 times as toxic as *S. aromaticum* and of higher order of toxicity compared with *Z. officinale*, *A. melegueta* and *X. aethiopica.* Levinson and Levinson (1998) reported that the application of some certain plant materials with grains can serve as fumigants. It has been reported that the sources of activity of these plant powders are the volatile constituents contained in the seeds. These volatile constituents are the essential oils. These essential oils contain a blend of volatile components of chemicals which cause a reversible reaction in herbivores when attacking plant species (Degenhardt *et al*., 2003). This blend of volatile oils can exert toxic, deterrent, antifeedant and repellent effects on insect herbivores (Pare and Tumlinson, 1999; Koul *et al*., 2008).

Table 4 shows that clove and WABP were able to completely prevent adult *S. zeamais* in laying eggs which would have caused progeny emergence. At the dosage rate of 0.5 – 2.0 g/10 g maize seed, no progeny emergence was observed or recorded. Statistically ($p \leq 0.5$) fewer number of progenies emerged in maize grains treated with Ethiopian pepper when compared to ginger and alligator pepper

Table 3: Acute toxicity of five different edible plant product powders (EPPP) exposed to adult *Sitophilus zeamais.*

Source of Edible Plant Product Powders	Plant Part Used	LC_{50} (g/10 g maize grain)	95% Fiducial Limits	Slope Values
Alligator pepper	Seed	3.038	1.732 – 10.128	1.568
Clove	Seed	0.694	0.376 – 0.927	2.383
Ethiopian pepper	Seed	3.300	2.056 – 5.711	2.050
Ginger	Rhizome	1.629	1.120 – 3.740	1.741
WABP*	Seed	0.427	0.101 – 0.649	2.224

*WABP = West African black pepper.

Table 4 Effect of edible plant product powders (EPPP) on *Sitophilus zeamais* infesting maize grains treated before infestation.

Dosage (g/10 g Seed)	Edible Plant Product Powders					
	Alligator pepper	Clove	Ethiopian pepper	Ginger	*WABP	Mean
Control	46.15	41.48	56.71	44. 81	47.50	47.33
0.50	24.87	0.00	9.03	24.90	0.00	11.76
1.00	19.38	0.00	12.70	20.58	0.00	10.53
1.50	13.97	0.00	5.48	20.85	0.00	8.06
2.00	3.83	0.00	3.85	12.13	0.00	3.96
Mean	21.64	8.30	17.55	24.65	9.50	

SED= 1.36; LSD= 3.87 (DOSAGE)

SED= 1.36; LSD= 3.87 (EPPP)

SED= 3.04; LSD= 8.65 (DOSAGE x EPPP)

*WABP = West African black pepper.

powders. There were no significance differences ($P \geq 0.05$) between progeny emergences in maize seeds treated with alligator pepper and ginger powders. The reactions of the dosage rates were dose dependent (Table 4). There were significant differences ($P \leq 0.05$) between the EPPP doses and the control. However, there were statistical significant differences ($P \leq 0.05$) between the dosage rates 0.5, 1.00 and 1.50 g when compared with each other. Application of EPPP at 2.0 g/10 g maize grain was statistically different from the control and the rest of the dosage rates. The table also showed that the effect of dosage rate and the EPPP were statistically significant ($P \leq 0.05$).

Table 5 showed the effect of the EPPP on stored maize grains that were already infested with adult *S. zeamais.* More significant ($P \leq 0.05$) number of progenies emerged from EPPP treated with ginger, Ethiopian pepper and Alligator pepper when compared to emergence in WABP and clove. There were no statistical significance ($P \leq 0.05$) differences when progeny emergence in maize grains treated with WABP and clove, were compared. These were however, significantly different from the rest of the EPPP. The use of higher concentration of EPPP also conferred a better protection on maize grains when compared with the control. There were however, no significance differences between application of EPPP at 2.0 and 1.00 g or 1.50 g/10 g maize grain. No statistical significant difference ($p \geq 0.05$) was also observed between EPPP applied at 0.50 and 1.00 g. The interaction effects of the EPPP and dosage rates were statistically significant ($p \geq 0.05$).

Table 5. Effect of edible plant product powders (EPPP) on *Sitophilus zeamais* infesting stored maize grains treated after infestation.

Dosage (g/10 g Seed)	Edible Plant Product Powders					
	Alligator Pepper	Clove	Ethiopian pepper	Ginger	*WABP	Mean
Control	49.42	57.28	49.22	54.24	48.90	51.81
0.50	23.86	18.32	26.05	30.55	18.38	23.43
1.00	22.72	13.32	24.74	28.03	12.55	20.27
1.50	21.09	12.37	17.23	25.17	11.48	17.47
2.00	20.64	10.86	17.79	22.56	10.07	16.38
Mean	27.01	22.43	27.01	32.11	27.27	

SED= 1.40; LSD= 3.98 (DOSAGE)

SED= 1.40; LSD= 3.98 (EPPP)

SED= 3.13; LSD= 8.91 (DOSAGE x EPPP)

*WABP = West African black pepper.

The results presented in Table 4 showed that edible plant product powders (EPPP) particularly *P. guineense* and *S. aromaticum* were effective in suppressing or completely inhibiting oviposition by *S. zeamais* while *X aethiopica, A. melegueta* and *Z. officinale,* were only effective albeit at high dosage rates. Also treatments with *P. guineense* and *S. aromaticum* completely inhibited adult emergence at dosage rates of 0.5, 1.0, 1.5 and 2.0 g/10 g, respectively. The differences exhibited in suppressing and/or inhibiting oviposition and adult emergence by the EPPP could be due to the different active ingredients (a.i) constituents contained in the EPPP. Earlier reports have shown that products from these plant materials either used as oil extracts or powders have been used in the control of stored products pests (Lale, 1992; Ajayi and Lale, 2000/2001; Ajayi and Lale, 2001; Ajayi and Wintola, 2006). The effectiveness of *P. guineense* is attributed to piperine, while the pungency and odour are due to volatile oil containing terpenes (Oliver-Bever, 1986). *S. aromaticum* has been reported to contain eugenol and caryophyline. Paradol has been reported to be the major constituents in *A. melegueta* (Lale, 1992).

The results Tables 4 and 5 showed the potentials of the EPPP as a good biopesticide for the control of *Sitophilus zeamais* infesting stored maize. It has been reported that powders of *P. guineense* and *S. aromaticum* at the rate of 1.0 g/20 g of cowpea seed was able to cause 100% egg mortality of *Callosobruchus maculatus,* thus inhibiting progeny emergence (Ajayi and Wintola, 2006). In another report powder of *A. melegueta*, *Eugenia aromaticum* (Syn. *S. aromaticum)* and *Piper umbellatum* (Syn. *P. guineense*) were reported to cause high mortalities of adult *Sitophilus oryzae* with subsequent result of lack of F_1 progeny (Lajide *et al.*, 1998).

The EPPP are very effective as a repellent, acute toxicity and as a suppression of oviposition progeny emergence of *S. zeamais* infesting stored maize grains. The results also showed that the treatment of maize grains with EPPP before infestation of *S. zeamais* was more effective in inhibiting progeny emergence compared with treatment after infestation. One important advantage of these plant products is that they can be afforded by resource-poor farmers and they already constitute an important component of the diet of Nigerians, and most Africans and Asians and they have form part of the international trade on spices. Hence, this would facilitate their adoption as grain protectants since the hazard of residues in stored food commodities usually associated with insecticides would be eliminated. The use of powders *P. guineense* and *S. aromaticum* may offer a greater protection for stored maize compared with the powders of *Z. officinale, A. melegueta* and *X. aethiopica*, respectively.

Dedication: The authors wish to dedicate this paper to the memory our former student Mr. Ishaya Agya Agidi**,** on the premises of whose undergraduate research project work the data in this paper are being presented for publication.

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