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Stability Analysis of Seed Germination and Field Emergence Performance of Tropical Rain-fed Sesame Genotypes

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ABSTRACT

The work was carried out to determine the stability of two seed quality traits (seed germination and field emergence) in 14 sesame genotypes that were grown in three plant population environments in Abeokuta, southwest Nigeria in each of two seasons. Seeds harvested from each environment were tested for these quality traits. Data obtained were subjected to analysis of variance of Finlay-Wilkinson regressions and stability analysis. Each genotype was defined by three stability parameters: (1) mean seed germination and field emergence over all environments, (2) the linear regression (b values) of genotype mean seed germination and field emergence in each environment, (3) the mean square deviation from the regression for each genotype (S^2d value). The genotypes varied considerably in the two seed quality traits and genotype x environment (GxE) interactions were significant. Regression coefficients ranged from 0.19 to 1.70 for seed germination and 0.14 to 3.01 for field emergence. Genotype 530-6-1 with a regression coefficient close to unit (b=1.03), smaller S^2d value and a relatively high seed germination of 79% had general adaptability and somehow averagely stable. The highest field emerging genotypes proved less stable and selection solely for high emergence could result in discarding many genotypes that were relatively better adapted to environmental changes. Genotypes 530-6-1, 73A-11 and C-K-2 were identified as desirable for seed production in all the three plant population environments. Genotypes 69B-88Z, Domu and 73A-97 were identified as desirable genotypes for cultivation in 133,333 plants ha $^{-1}$ environment, C-K-2 in 166,667 plants ha $^{-1}$ environment and 93A-97, 73A-11, 69B-88Z and C-K-2 in 266,667 plants ha $^{-1}$ environment to obtain seed of high and stable germination and emergence. These genotypes were superior in seed quality and therefore deserve a place in commercial seed production and future seed improvement strategies. [Report and Opinion. 2009;1(5):1-8). (ISSN: 1553-9873].

Keywords: environment, interaction, plant population, seed quality,

Introduction

Seed quality is defined as a standard of excellence in certain characters or attributes that will determine the performance of the seed when sown or stored (Hampton, 2002). It relates to the characteristics of seeds which result in the high field performance and eventually high seed/grain yield. Seed germination and field emergence have been identified as good indicators of seed quality in different crops.

Most of the quality characteristics are polygenically inherited, and will therefore be influenced by the environment to a large extent (Labuschangne *et al*., 2002). Studies have shown that seed quality can be largely influenced by a wide range of environmental factors during seed production, harvesting, processing, storage and treatments such as seed priming (Tektrony *et al*., 1980; McDonald, 2000; Adebisi and Ojo, 2001; Tesnier, 2002; Adebisi and Ajala, 2007). Those factors of the production environment which dictate the quality of seeds produced include temperature, available moisture during seed development and maturation, incidence of diseases and pests in the field and at storage, management practices, harvest and post-harvest seed handling (Tekrony *et al*., 1980; Adeyemo *et al*., 1998; Adebisi and Ojo, 2001).

Different attempts have been made to solve the problems created by genotype x environment interactions (Hanson *et al*., 1956; Comstock and Moll, 1963). Most of the estimates, however, only provide information on their existence and magnitude, but give no measurements of the individual genotype. Selection of stable genotype that performs consistently across environments can reduce the magnitude of these interactions. Besides, stability of sesame performance is of special importance under rain-fed conditions in developing countries where environmental conditions varied considerably and the technologies of modifying the environments are far from adequate (Adebisi, 2004). Interest has been focused on the regression analysis, an approach originally proposed by Yates and Cochram (1938) and later modified by Finlay and Wilkinson (1963) and Eberhart and Russel (1966). Regression analysis has been widely used in comparing and measuring genotypic performances of common bean (Beaver *et al.*, 1985), Soybean (Ojo,

2002 and Ojo *et al*., 2002), cashew (Adebola and Esan, 2002), navy bean (Gebeyehu and Assefa, 2003) and sesame (Adebisi, 2004)

Most of the sesame (*Sesamum indicum* L.) genotypes grown in the South-west Nigeria were selected based only on their desirable seed weight or yield per hectare with little or no reference to stability of seed quality performance. This has resulted in poor yield and quality of seed obtainable. Although sesame is grown in diverse plant population environments in Nigeria, there is currently no information on the seed quality stability and response of different tropical sesame genotypes under these environments. There is the need to identify outstanding genotypes with stable, desirable and superior seed quality for the farmers.

A genotype is stable if, at a given location or plant population it exhibits very little fluctuation in seed quality from year to year. An ideal sesame selection (genotype) is therefore one that combines high seed quality and stable performance in most of the ecological environments where it is cultivated. Therefore, the present work was conducted to determine the stability of seed germination and field emergence performance in some tropical rain-fed sesame genotypes grown in south-west Nigeria under three plant population environments and identify genotypes that performed well under such environments.

Materials and Methods

Fourteen sesame genotypes sourced from the National Cereals Research Institute, Badeggi, Niger State, Nigeria were evaluated in trials conducted at the Teaching and Research Farm of the University of Agriculture, Abeokuta (7^o15'N, 3^o25'E). Seeds of the 14 sesame genotypes were grown under three plant populations during the rainy seasons of 2001 and 2002. The treatments formed experimental environments as follows: Environment 1 = 50 cm x 15 cm (133,333 plants ha^{-1}), Environment 2 = 60 cm by 10cm (166,667 plants ha^{-1} and Environment 3 = 75 cm x 5 cm (266,667 plants ha^{-1}). The plant populations and seasons, therefore, constituted six environments.

The experimental fields were well-drained sandy-loamy soil with a pH range of 6.81 to 7.80, nitrogen status between 0.07% and 0.14%, organic matter between 1.42% and 2.86% and carbon status between 0.82% and 1.66 %. The average rainfall for the two seasons ranged from 500 mm annum $^{-1}$ in 2001 to about 800 mm $annum^{-1}$ in 2002. At each plant population and in each season, the 14 entries were arranged in randomized complete blocks with three replications. Sowing was done by hand in four-row-plots of 3 m long and spaced 50 cm x 15 cm, 60 cm x 10 cm and 75 cm x 5 cm. Seeds were mixed with sand and hand drilled while seedlings were thinned at 3 weeks after sowing to about 15 cm, 10 cm and 5 cm between plants. Following thinning, a post emergence fertilizer application of NPK 15:15:15 was applied by drilling at the rate of 60kgN, 30kg P_20_5 and 50kg K_20 ha^{-1}. Weeding was carried out twice before and after fertilizer application.

Seeds harvested from each of the environments were evaluated in the seed laboratory for seed germination and field emergence thus:

Seed germination: The test was performed according to ISTA (1995). Three 100-seed replicates of each genotype were germinated in 11cm diameter petri dishes inside a moistened paper towels with 5ml of distilled water. The petri dishes were arranged inside an incubator at 30^0C temperature in a completely randomized design. After seven days of germination, the proportion of germinated seed (visibly emerged normal radicle) was expressed as normal germination percentage.

Field emergence: Four sub samples of 50 seeds for each genotype under each environment were hand-sown in furrows of 2.0m, 0.30m apart and 0.05m deep in the field. Soil medium was kept sufficiently wet for emergence. The number of emerged seedlings was counted at 14 days after sowing and expressed as percentage of seed sown.

Data Analysis

Data generated were firstly transformed using angular transformation (arcsine). and then subjected to analysis of variance of Finlay-Wilkinson regressions using GENSTAT (2001) 10.0 statistical package.

Stability parameters for each genotype were determined using the regression procedure of Eberhart and Russel (1966). Each genotype was defined by three values: (1) mean seed germination and field emergence over all environments, (2) the linear regression (b values) of genotype mean seed germination and field emergence in each environment, (3) the mean square deviation from the regression for each genotype (S^2d value). Significance of regression co-efficient (b-values) was tested by the student's t-test (Steel *et al*., 1997). For the regression analysis of variance, the residuals from the combined analysis of variance were used as a polled error to test the significance of the S^2d values (Osman, 1991). A significant F-value would indicate that S^2d was significantly different from zero. Co-efficients of determination (r^2 values) were computed from individual linear regression analysis (Pinthus, 1973).

Stimulation of current experiment by varying the number of plant density was used to determine the most efficient plant density for sesame seed quality testing under rain fed tropical conditions.

Results

Results of analysis of variance of Finlay-Wilkinson regressions for seed germination and field emergence are presented in Table 1. There were high significant mean squares for environment and genotype x environment interaction for seed germination and field emergence. Genotype effects were highly significant for seed germination and field emergence.

Stability parameters of seed germination of 14 sesame genotypes evaluated in six environments are presented in Table 2. Regression co-efficients ranged from 0.19 (for genotype 73A-97) to 1.70 (for genotype Type A). Six genotypes (Goza, Type-A, E8, Domu, C-K-2 and 530-3) had regression co-efficients greater than 1.0. One of these genotypes (C-K-2) had higher seed germination than the mean of all the genotypes. However, seven genotypes (73A-97, Pbtil No1, 69B-88Z, 73A-94, 73A-11, 93A-97 and Yandev 55) had regression co-efficients less than 1.0. Genotype 530-6-1 had regression co-efficient close to unit (b = 1.03).

Results in Table 3 show the stability parameters of field emergence of 14 sesame genotypes evaluated across six plant population environments. Regression co-efficients for field emergence trait ranged from 0.14 (for Pbtil No1) to 3.01 (for 73A-94). Eight genotypes (93A-97, 93A-11, Type-A, 530-6-1, 73A-94, Domu, 73A-97 and 530-3) had regression co-efficients higher than 1.0. Four of these genotypes (73A-11, 530-6-1, 73A-94 and 73A-97) had higher field emergence than the mean of all the genotypes. Regression co-efficients of Goza, 69B-88Z, Yandev 55, E8, C-K-2 and Pbtil No1 were less than 1.0 with field emergence below the mean of all the genotypes except for Yandev 55, 69B-88Z and C-K-2 which had higher mean than mean of all the genotypes.

As shown in Table 4, seed germination of the sesame genotypes showed significant differences in each of the three plant population environments. Genotypes 69B-88Z (78%), 530-6-1 (77%) and Domu (77%) as well as 73A-97 (76%) had higher seed germination at 133,333 plants ha^{-1}. Similarly, C-K-2 (80%), 73A-11 (78%), 93A-97 (78%), 530-6-1 (77%) and 73A-94 (77%) recorded remarkably higher seed germination at 166,667 plant ha^{-1} while 73A-97, Yandev 55, C-K-2, 73A-11 and 530-6-1 with seed germination above 80% were among genotypes with significant higher seed germination at 266,667 plant ha^{-1}.

In Table 5, 73A-97, 73A-94, Yandev 55, 73A-11, 69B-88Z and 530-6-1 were among genotypes that had significant greater field emergence at 133,333 plants ha^{-1} while Pbtill No1 (85%) followed by C-K-2 (75%) and E8 (71%) recorded significant higher emergence at 166,667 plants ha^{-1}. At 266,667 plants ha^{-1}, 73A-97, 5306-1, C-K-2and 93A-97 and 73A-11had significant higher emergence of 73, 71, 70, 69 and 69%, respectively.

Table 1: Analysis of variance of Finlay-Wilkinson regressions for seed germination and field emergence over 14 sesame genotypes in six environments.

Source of variation	DF	Mean Square Values	
		Seed germination	Field emergence
Replication	12	6.69	34.37
Genotype (Gen.)	13	195.61**	267.12**
Environment (Env) (Linear)	5	1069.90**	266.86**
Gen.xEnv.(Linear)		154.68**	147.49**
Pooled Error	156	11.33	18.08

** Significant at 0.01 level of probability ns = not significant

Table 2. Mean seed germination and estimates of stability parameters in 14 sesame genotypes evaluated over six environments

Genotype	[+] Mean seed germination (%)	R^2	FWb	S^2d	T
Yandev 55	77[a]	0.22	0.69[ns]	0.64[ns]	1.07
93A-97	76[a]	0.23	0.57[ns]	0.52[ns]	1.09
Goza	68[d]	0.44	1.47[ns]	0.83[ns]	1.78
Type-A	70[cd]	0.68	1.70*	0.59[ns]	2.88
73A-11	77[a]	0.56	0.79[ns]	0.35[ns]	2.25
530-6-1	79[a]	0.82	1.03**	0.24[ns]	4.27
73A-94	73[bc]	0.53	0.84[ns]	0.40[ns]	0.17
69B-88Z	76[ab]	0.60	0.98[ns]	0.40[ns]	2.43
E8	71[c]	0.91	2.31**	0.37[ns]	6.22
Domu	72[c]	0.41	1.58**	0.94[ns]	1.68
73A-97	78[a]	0.21	0.19[ns]	0.52[ns]	0.36
C-K-2	77[a]	0.38	1.12[ns]	0.71[ns]	1.56
530-3	72[c]	0.53	1.67[ns]	0.08[ns]	2.11
Pbtil No1	71[cd]	0.07	0.21[ns]	0.38[ns]	0.56
Mean	74		1.00		

Mean values within a column with a letter superscript in common are not significantly different at $P < 0.05$
*, ** FWb value significantly different at 5% and 1% levels of probability respectively
FWb: Finlay-Wilkinson regression co-efficient,
R^2 = coefficient of determination
S^2d = Mean square deviation from the regression
t = 't' test value
[+] Mean standard germination after angular transformation

Table 3. Mean field emergence and estimates of stability parameters in 14 sesame genotypes evaluated over six plant population environments

Genotype	[+] Mean Field emergence (%)	R^2	FWb	S^2d	T
Yandev 55	67[ab]	0.11	0.90[ns]	1.27[ns]	0.71
93A-97	62[bc]	0.38	2.09[ns]	1.33[ns]	1.58
Goza	58[c]	0.01	0.21[ns]	0.94[ns]	0.22
Type-A	59[bc]	0.36	2.17[ns]	1.44[ns]	1.50
73A-11	68[ab]	0.89	1.40**	0.23[ns]	5.98
530-6-1	66[b]	0.68	2.29*	0.79[ns]	2.91
73A-94	66[b]	0.73	3.01*	0.93[ns]	3.25
69B-88Z	66[b]	0.01	0.17[ns]	0.90[ns]	0.18
E8	63[bc]	0.01	0.39[ns]	1.78[ns]	0.22
Domu	64[bc]	0.78	2.29**	0.60[ns]	3.77
73A-97	69[a]	0.72	2.37*	0.74[ns]	3.21
C-K-2	71[a]	0.02	0.35*	1.32[ns]	0.26
530-3	63[bc]	0.66	2.80*	1.02[ns]	2.76
Pbtil No1	61[c]	0.00	0.14[ns]	1.34[ns]	0.10
Mean	65		1.00		

Mean values within a column with a letter superscript in common are not significantly different at $P < 0.05$
*, ** FWb value significantly different at 5% and 1% levels of probability respectively
FWb: Finlay-Wilkinson regression co-efficient,
R^2 = coefficient of determination
S^2d = Mean square deviation from the regression
t = 't' test value
[+] Mean field emergence after angular transformation

Table 4. Performance of seed germination under three plant population environments over two cropping seasons.

Genotype	Seed germination (%) 133,333 plants ha^{-1}	166,667 plants ha^{-1}	266,667 plants ha^{-1}
Yandev 55	72	74	84
93A-97	72	78	78
Goza	70	71	54
Type A	70	75	85
73 A-11	73	78	80
530-6-1	77	77	82
73A-94	69	77	74
69B-88Z	78	73	75
E8	71	73	68
Domu	77	75	65
73A-97	76	76	84
C-K-Z	74	80	80
530-3	70	76	71
Pbtil No1	71	70	74
Mean	73	75	75
Lsd(0.05)	5.19	5.52	5.45

Data presented according to method of Choo *et al.* (1984) of determination of stability of performance

Table 5: Performance of field emergence under three plant population environments over two cropping seasons.

Genotype	Field emergence (%) 133,333 plants ha^{-1}	166,667 plants ha^{-1}	266,667 plants ha^{-1}
Yandev 55	69	68	64
93A-97	61	55	69
Goza	60	61	56
Type A	51	63	63
73 A-11	68	68	69
530-6-1	67	67	71
73A-94	71	61	65
69B-88Z	68	62	68
E8	53	71	54
Domu	65	63	63
73A-97	71	63	73
C-K-Z	66	75	70
530-3	59	62	66
Pbtil No1	60	85	65
Mean	64	66	65
Lsd(0.05)	4.41	5.02	5.19

Data presented according to method of Choo *et al.* (1984) of determination of stability of performance

Discussion

The results of joint regression analysis revealed that the GXE (linear) effect due to environment showed significant differences between regression co-efficients pertaining to the regression of genotype seed germination and field emergence on environmental seed germination and field emergence. The result revealed differences among slopes of regression lines and the regression model was adequate in explaining stability of the 14 sesame genotypes in respect of their seed quality (seed germination and field emergence). These observations are in agreement with that reported by Adebisi and Ajala (2006) for sesame seed yield in south- west Nigeria.

In this study, the coefficients of determination (R^2) ranged from 0..07 to 0.91 Since the environmental sum of squares contributed to the

regression sum of squares, Moll *et al*., 1978 and Osman (1991) showed serious concern in the interpretation of R^2 values. Osman (1991) reported that linear regressions accounted for 76-99% of the variation in sesame seed yield. Similarly, Adebisi and Ajala (2006) observed that linear regression accounted for 0.65-1.25 of the variation in seed yield of Nigerian sesame genotypes. In this study, linear regressions contributed as much as between 07 and 91% of the variation in seed germination and between 01 and 89% in field emergence. The significant differences in b values suggested that all the 14 sesame genotypes responded differently to the different plant population environments. Variability in environments was an important factor and largely determined the usefulness of b values (Pfahler and Linskens, 1979).

The stability result of seed germination indicated that Goza, Type-A, 530-6-1, E8, Domu, C-K-2 and 530-3 had regression coefficients greater than 1.0, they were, therefore, sensitive to environmental changes in respect of seed germination. However, one of these genotypes (C-K-2) with higher seed germination than the overall genotype mean suggests that it could be recommended for cultivation under productive environments for higher seed germination. Genotypes 73A-97, Pbtil No1, 69B-88Z, 73A-94, 73A-11, 73A-97 and Yandev 55 had regression co-efficients less than 1.0. These genotypes were relatively better adapted to poor environment and were insensitive to environmental changes in respect of seed germination. Such genotypes could be recommended only for cultivation in unfavourable conditions. Also genotype 530-6-1 with regression co-efficient close to unit (b = 1.03) had general adaptability and somehow averagely stable.

For field emergence performance, genotypes 73A-11, Type-A, 530-6-1, 73A-94, Domu, 73A-97 and 530-3 had regression co-efficients above 1.0, and they were therefore sensitive to environmental changes for field emergence. Four of these genotypes (73A-11, 530-6-1, 73A-94 and 73A-97) recorded higher field emergence than the genotype mean, and hence, could be recommended for production under productive environments. Conversely, field emergence of six genotypes (Goza, 69B-88Z, Yander 55, E8, C-K-2 and Pbtil No1) had regression co-efficient values less than 1.0, with mean emergence of either below or above genotype mean, hence, they were relatively better adapted to environmental changes and could be suggested for cultivation in unfavourable conditions, without any adverse effect on field emergence.

According to Eberhart and Russel (1966), a genotype considered as stable should meet criteria of high mean performance, with b equal to unity and S^2d approaching zero. Using these criteria, seed germination of genotype 530-6-1 with regression coefficients of 1.03, S^2d approaching zero and with relatively high seed germination of 78.50% could be considered widely adapted and stable. It has the ability to express its germination potential when produced in a range of environmental conditions. The highest field emerging genotypes proved less stable and selection solely for high emergence could result in discarding many genotypes that were relatively better adapted to environmental changes.

In a similar vein, Choo *et al*. (1984) described a desirable genotype as one with high mean, at least average performance, in all environments and an undesirable genotype as having either a low mean performance or below-average performance in some environments. Following Choo *et al.* (1984) criteria and defining high mean seed germination as at least 5% above the grand mean (Table 4), only 530-6-1 showed itself to be desirable in each of the plant population environments. However, for field emergence (Table 5), the performance at individual plant population environment indicated that 73A-11 and C-K-2 maintained above average emergence in each of the three plant population environments evaluated.

The method of Choo *et al*. (1984) coupled with the regression analysis have jointly pointed out genotypes 530-6-1, and 73A-11 and C-K-2 as desirable genotypes that will give good germination and field emergence, respectively over an array of environments encountered in the south-west of Nigeria and similar ecologies. Moreover, when applied to individual plant population environment, the method of Choo *et al*. (1984) pointed out 69B-88Z, Domu and 73A-97 as being most suitable for seed production in 133,333 plants ha^{-1} environment and genotypes 73A-11 and C-K-2 in 166,667 plants ha^{-1} environment. However, genotypes 93A-97, 73A-11, 73A-97, 69B-88Z and C-K-2 would be appropriate in 266,667 plants ha^{-1} environment to obtain stable and high seed germination and emergence.

Conclusion

The investigation of stability of sesame genotypes clearly showed that most of the test genotypes were sensitive to production environments. Hence, their wider adaptability, stability and general performance to the fluctuating growing conditions within and across plant population environments were considerably lowered. The stability analysis provides meaningful information regarding stability and consistency of seed quality performance of sesame genotypes across different environments. These genotypes can be obtained from the University of Agriculture, Abeokuta, Nigeria and National

Cereal Research Institute (NCRI), Badeggi, Nigeria. The identified genotypes may be used as parents in future sesame crop improvement programmes. Sesame seed must be tested for germination and vigour in different environments to determine the favourable conditions for sesame seed production, as discussed by Heydecker (1972); Dickson (1980); Odiemah (1991) and Adebisi (2004).

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7/1/2009

A Note on Micro Tuber Seed Production of Potato: Necessitate Step for Uttarakhand Hills

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Abstract: This article gives a note on micro tuber seed production of potato. [Report and Opinion. 2009;1(5):9-11). (ISSN: 1553-9873].

Keywords: micro tuber; seed production; potato

Traditional agriculture is not economical for the hill areas of the India because not only the yields low but the crop also takes longer time to mature. It has been observed that there is a drastic improvement and change in the socio-economic status of the hill people particularly in those areas where the pockets and valleys have bean exploited. Production and consumption of vegetables is directly related to the improvement of the socio-economic status of the hill people. Potatoes have better food value and their role in the daily diet of human beings as a protective food is well recognized. Most of the farmers, food processors and scientists prefer to concentrate on production nature than on planning for crop. The same story is repeated with potato cultivation, too. The countrywide efforts have been on to dawn a revolution. This has emerged as one of the major agricultural venture in the nineties. Since good quality seed supply is rationale with the production and demand of the same and the seed production is covered under seed act etc. which does cover planting material initially supplied by the breeder's and followed by on site nursery certification as per technical standard fixed.

Since antiquities the traditional farmers of Uttarakhand were growing variety of vegetables in kitchen garden based farming system or backyard farming system. The main crops taken during past were comprising of Kachalu, Arbi, Turai, Lauki, Pumpkins, Karelas, Kakodas, Ishkush, Snake Gourds, Kakri and Kheera, Petha, Lady Finger, Brinjal during rainy seasons and Carrots, Radish, Pindalu, Methy, Palak, Chaulai, Rai, Onion in winters. Though some spices and condiments like Chilies, Dhania, Garlic, Ginger, Turmeric, Large cardamoms, Saunf, Tulasi, etc. were also in cultivation. If controversial debate to trace the actual date of potato cultivation in Uttarakhand is put to be immaterial, potato is being grown here for the last 500 years, when tribes were cooking it raw in dung cake fires during their religious fasts along with yams (though the introduction of crop is believed in the country after year 1830 by Sulavan and after 1832 Captain Tichmond in Uttarakhand. Britishers believed that England potato is much superior to that of local (Indian) varieties.

The potato is one of the most important food crops both in developed as well as developing countries. Due to its diversified uses in developed countries as food, feed raw material for producing starch. The potato was generally regarded to be a crop suited for western world. Potato is next only to rice, wheat and maize in cultivation in India. Next to cereals potato is the only crop, which could supplement the need of the food of the country. It is potentially a crop, which can be harvested, and the tuber can be consumed any time after sixty days of planting. As a source of energy it surpasses cereals like wheat and rice (Das; 1999).

In India potato is grown in almost all the states. Nearly 80% of the crop is grown in Indo-Gangetic plains comprising Punjab, Haryana, Uttar Pradesh, Bihar and West Bengal. Its world average yield is 16.1 tonnes/ha and per caput availability is 50.5 kg/year (Prasad; 2004). In all potato growing regions the availability of high quality clean seed tuber has been the most limiting owing to the conventional clonal propagation that favors disease build-up (Plate:1-a and b) that drastically reduces yield.


a


b

Plate 1: (a) and **(b) -** Late Blight disease

However, the recent advancement in tissue culture and the flexibility of organ development in potato allows for alternative methods of propagation through *in vitro* techniques. Potato seed production programmes in many countries have been boosted by using these techniques. In recent years the first multiplication steps in seed production programmes are speeded up by using *in*

vitro plantlets, Microtubers or mini tubers (Hussey and Stacey 1981).

Potato can supplements the food needs of the country in a substantial way. It produces dry matter food, well-balanced protein and more calories from unit area of land and times then other major food crops. It contains practically all the essential dietary constituents like cereals, carbohydrates that are the major constituents of potato. Besides it contains essential nutrients as proteins and minerals like calcium, phosphorus, iron and vitamins: B1, B2, B6 and C, (Thamburaj and Singh; 2001).

Successful cultivation of seed potato depends upon the availability of disease free seed, soil, moisture, plant protection measures, low temperature, short days conditions during tuberization phase, resulting rapid bulking rate. Potato plant is very sensitive to ecological factor such as temperature, rainfall and photoperiod. (Singh; 2002) Conventional propagation of potato is done vegetatively using seed tubers and ensures uniformity of the crop in terms of growth and yield, but results in degeneration of the crop due to virus infection, the rate of degeneration 398 varying from place to place and from cropping season to cropping season. The viruses are transmitted through different ways including through planting infected tubers. If the seed stock is not maintained well or frequently replaced with fresh ones, the virus infiltration can reach up to 100% in 3 - 4 successive crop seasons resulting in almost half or one third yields. This is the major problem faced by seed producers.

Almost half a century has passed since in vitro tubers (microtubers) were first described in potato, but their adoption as a seed propagule has been uneven globally. Consensus is lacking regarding optimal production practices for microtubers and their relative productivity in relation to other propagules for minituber production. There is significant uncertainty regarding the utility of microtubers for evaluation of agronomic characters. However, the application of microtubers in germplasm conservation is widely accepted. Microtubers are produced in vitro in a plethora of different growing systems with varying environment, media constituents, and storage intervals (Plate: 2- a and b). Many of the interactions between growth parameters in vitro and subsequent productivity appear to be genotype-specific. Accordingly, microtubers come in different sizes, have different dormancy requirements, and differ widely in relative growth potential and productivity. Despite these differences, there is evidence for strong analogies in growth responses between field-grown tubers and microtubers (Badoni and Chauhan, 2009).

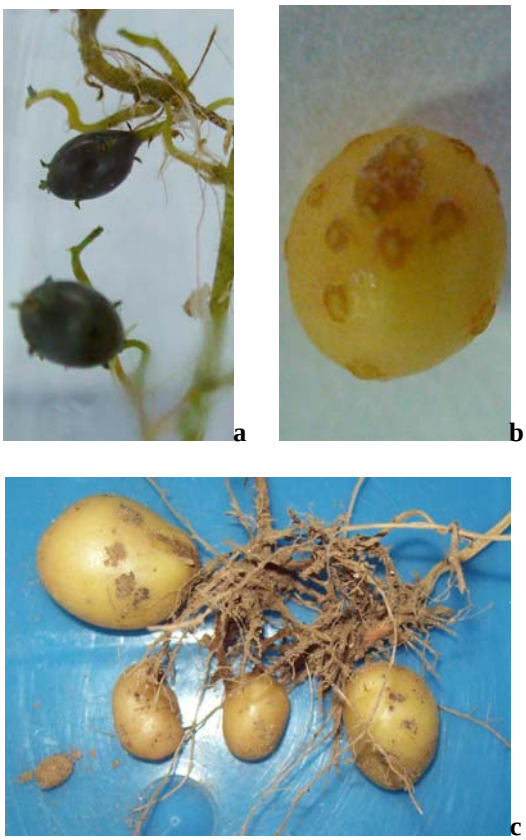

Plate-2: (a) and **(b)** Microtubers and **(c)** minitubers

The use of microtuber technology in seed tuber production, breeding programs, germplasm conservation, and research appears to have enormous potential. Microtubers are utilized for minituber (small tubers produced from in-vitro-produced propagules; Plate: 2- c) production in greenhouses or screenhouses and, less commonly, are directly field-planted. Wherever microtuber and minituber production technologies have been implemented, they have halved the field time necessary to supply commercial growers (3 or 4 years compared with 7 or more years), and greatly improved seed tuber quality (fewer viral, bacterial, fungal problems) (Donnelly, Danielle J, Coleman, Warren K, Coleman, Shirlyn E, 2003).

To large production of clonal material i.e., to produce the uniform, identical seed material of potato, micro propagation is the better alternative over to conventional propagation of potato. The in vitro propagation method is most suitable alternative to produce Microtuber seed material of potato. By using the technique, which involves low cost components, the large scale clonal material can be achieved in short time duration.

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7/5/2009

Importance and Problems in Natural Regeneration of *Spondias pinnata*

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Abstract: This article describes importance and problems in natural regeneration of *Spondias pinnata*. [Report and Opinion. 2009;1(5):12-13). (ISSN: 1553-9873].

Keywords: Importance and Problems; Natural Regeneration; *Spondias pinnata*

Spondias pinnata

Common name:	Wild mango, Hog-plum, Amara
Altitude:	1500 m.
Distribution:	Indian Himalayas, Andaman Island, Srilanka, Myanmar Thailand, Malaysia and china
Description	Deciduous in nature and accomplish, a height of 9 m to 18 m. Bark thick aromatic

Importance of *Spondias Pinnata*

- Its wood is employed for packing cases, tea chests and match – splints.
- The fruits are eaten as a vegetable when green and as a fruit when ripe. Fruits are very nutritious and rich in vitamin A, minerals and iron content.
- The bark is useful in dysentery and diarrhea, and is also given to prevent vomiting.
- The root is considered useful in regulating menstruation.
- The plant is reported to have anti-tubercular properties.
- The leaves are aromatic, acidic and astringent. They are used for flavoring.
- The flowers are sour and used in curry as a flavoring and also eaten raw.
- Through value addition of this wild edible fruit tree plant the local people make chutney, jam and pickle. By production and marketing of these products, the local people may increase their socio-economic status.

(a) Immature Fruits

(b) Mature Fruits

Value Addition

- Wild fruits of many plant species have played a prominent role in the diet and medicine of human beings, particularly in the tribal and rural areas of the country, for thousands of years.
- If educated/uneducated and unemployed youth of this region engage themselves fully in the preparation of quality food and other related products from wild edible fruits like species as a source of income, the threat of unemployment could certainly be minimized.
- There is a great scope for enhancing the acceptability of wild edibles as income generating resources for the hill communities and initiating the potential plant resources for human consumption.

Problems in Natural Regeneration

- Hard seed coat, which creates a problem in seed germination.
- Commercial exploitation of the species because of the fruit and for other medicinal purposes by the locals.
- Lack of traditional knowledge know-how about the importance of the species and various uses.
- Seeds are damage because of the consumption of fruit by birds, monkey and other animals.
- Seeds are prone to attack by various pests, pathogen and insects in nature.
- *Lantana* might be creating inhibitory effect on both, the soil and species.
- The radicle is apt to dry, if not covered, or may be eaten by birds and insects.

There are many problems in the natural regeneration i.e. the hard seed coat create a problem in natural regeneration. Commercial exploitation and lack of traditional knowledge is also a very important problem. S*pondias pinnata* bear maximum pressure in natural habitat due to their higher demand for domestic consumption by locals. Attempts will be made to utilize this species as a source of income, particularly for poor rural inhabitants and unemployed youth of the regions by making a variety of values added edible products, such as juice, squash etc. Sustainable utilization and conservation of this plant as gained considerably importance, since it is also the key element of biodiversity.

7/6/2009

Technical Report

Effect of Velocity of Cleaning Pigs on the Efficiency of Fluid Delivery in the Pigged Pipeline System

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Abstract: Studies have been carried out to investigate the effect of velocity of cleaning Pigs on the efficiency of fluid delivery in pigged pipeline system. The results of the investigation indicate that increase in the velocity of the Pig increases the acceleration of the Pig leading to increase in the force exerted on the wall of the pipe. This invariably results to increased dislodgment and removal of corrosion products, films, particles, wax and other unwanted debris accumulated within the pipeline. It was observed that the efficiency of fluid flow through pigged pipelines is dependent on the velocity of fluid flow (after pigging) which in turn depends on the velocity with which the Pig ran during the cleaning process. [Report and Opinion. 2009;1(5):14-18]. (ISSN: 1553-9873).

Keywords: Keywords: Velocity, Cleaning Pigs, Efficiency, Fluid Delivery, Pipeline System.

1. Introduction

Deposition onto a metal surface, of particles normally suspended in solution has been reported (Ijomah,1991) as biofouling. The colloidal matter could come from so many sources both internal and external. They could enter the process through make up water, especially the untreated surface water. It could be scrubbed from the air as in open recirculation systems and could also come from organic deterioration products. These particles tend to take up a charge opposite to that of the metal surface, and fouling results causing a decline in heat transfer (Ijomah,1991). Algae, i.e., chlorophyll-containing organisms that need light for growth, are usually present in industrial cooling towers. If allowed unchecked, they are capable of producing great masses of material whose weight could endanger the structure and whose mass impedes air and water flow. Even small growths could slough off and be carried into circulating stream as fouling matter.

Slimes normally contain fungi, yeast, bacteria and trapped quantities of organic and inorganic matter (Ijomal,1991). Slimes forming bacteria are usually encapsulated in this gelatinous mass. Alive, they attach themselves to steel surfaces and grow to restrict heat transfer. In extreme cases, they can also restrict water flow and the deposits also set up concentration cells, causing corrosion (Ijomah,1991).

Aerobic iron bacteria can oxidize ferrous ions in solution to the ferric state and thus effect the precipitation of ferric hydroxide (Crook,1986). These organisms are common inhabitants of springs and may find their way into water, oil and gas pipes. Precipitated ferric hydroxides can build up on the internal surface of a pipe to form hard excrescences known as tubercles, which are firmly adherent to the metal surface and resist fluid flow (Loverell,1989). The tubercle shields the surface of the pipe from contact with dissolved oxygen (Loverell,1989). Hence, the metal at the base of the tubercle becomes anodic to those parts not covered by the deposit. The problem is often intensified by the fact that sulphate reducing bacteria takes advantage of the anaerobiosis created by the tubercles to make their input to the total corrosion.

Most instances of corrosion in the absence of oxygen have been attributed to the sulphate reducing bacteria (SRB), of genera; disulfovibrio and disulfotomaculum (Booth,1985). Typical examples of such an environment are natural water-logged soils and waters heavily polluted with organic matter. These microbes are obligate anaerobes, i.e, they will not grow in the presence of even traces of oxygen, but usually grow well at pH between 5 and 9 and temperatures between 25 and 44^0C, although some strains (disulfotomaculum nigrificans) are able to withstand higher temperatures (Ijomah,1991). They utilize hydrogen or some reducing substances for their life process. The corrosion is often localized and is generally characterized by a black corrosion product with a strong smell of hydrogen sulphide (H_2S) (Ijomah,1991). Sulphate reducing bacteria can cause serious damage in buried pipes, central heating installations, heat exchangers and cooling towers.

Mechanism of attack by sulphate reducing bacteria

Sulphate reducing bacteria have been found (Ijomah,1991) to utilize hydrogen for their metabolism. It then follows that for an iron or steel structure which has become polarized through the formation of hydrogen at the cathode and Fe^{2+} at the anode, the bacteria obtain their necessary hydrogen at the cathodic site, thus depolarizing the cathode. The S^{2-} ions arising from the sulphate reduction process then combines with Fe^{2+} ions to form ferrous sulphide (FeS). Hence, the anode reaction is also polarized, allowing the attack to proceed unhindered. Mathematically, the reaction is believed to follow the scheme (Ijomah,1991):

$$Fe = Fe^{2+} + 2e \quad \text{(anodic reaction)} \qquad (1)$$

$$2H^{+} + 2e = \text{(cathodic reaction)} \qquad (2)$$

$$SO^{2-}_{4} + 8H = S^{2-} + 4H_2O \qquad (3)$$

(cathode depolarization by SRB)

$$Fe^{2+} + S^{2-} = FeS \quad \text{(corrosion product)} \qquad (4)$$

$$FeS + 2H_2O = H_2S + Fe(OH)_2 \qquad (5)$$

(corrosion product)

It was found (Ijomah,1991) that when microbial growth occurs on a structure liable to corrosion such as pipeline, a differential aeration cell is usually set up between those parts of the structure where oxygen supply has been is depleted and those parts where micro-organisms are not active. The oxygen depleted regions will be anodic to the rest and will therefore become centre for active metal loss.

Observations show that pigging in pipelines is inevitable in order to achieve very high level of pipeline integrity by elimination of various kinds of corrosion products, films, particles and wax that accumulate in the pipes. It was also observed that the efficiency of fluid delivery depends mainly on the level of cleanliness achievable within the internal area of the pipelines, which in turn depends on the effectiveness of the pigging process.

The present work is aimed at investigating the effect of velocity of cleaning pigs on the efficiency of fluid delivery in the pigged pipeline system. In this work, pigs were used to clean the pipes of various kinds of corrosion products, films, particles and wax accumulated by the activities of sulphate reducing bacteria and environmental influences so as to maintain high efficiency of fluid flow through the pipes.

2. Materials and methods

In this study, work was carried out on three major oil pipelines; PPL 1, PPL 2, and PPL 3 at offshore platforms in Akwa Ibom State, having been certified infested with sulphate reducing bacteria (following preliminary phynotipic examination carried out) and observed to contain deposits of wax and particles. Varied velocities of Pig were recorded by varying the volume fluid flow and the pressure within the internal area of the pipe. The mass of the debris dislodged from the pipe during the pigging process was determined for each velocity of Pig considered. The time elapse (in seconds) during which the pigging process occurred was recorded. Details of the stages of the experiments and techniques used are as stated in previous report (Nwoye,2002).

3. Results and discussion

As Pig moves at increased velocity V, it exerts increased force called Force of Erasure on the wall of the pipe in accordance with the equation (Okeke,1987);

$$F = ma \qquad (6)$$

Where

$$a = \left(\frac{V - U}{t}\right) \text{(Okeke,1987)} \qquad (7)$$

Therefore, substituting equation (7) into equation (6), it reduces to;

$$F = m\left(\frac{V - U}{t}\right) \qquad (8)$$

Where

F = Force (N)
m = Mass of Pig (53.8 Kg)
a = Acceleration of the Pig (m/s^2)
V = Final velocity of Pig (m/s)
U = Initial velocity of Pig at time t = 0 (m/s)
T = Time elapse between the beginning and end of the pigging process

The increased force was observed to cause increased dislodgment and removal of the corrosion products, films, particles and wax accumulated on the pipeline. Equation (7) shows that when the velocity of the Pig increases, the acceleration of the Pig increases. Equation (8) shows that this results to increase in the force exerted on the wall of the pipe since the mass of the Pig is constant. Similarly, increase in velocity has been reported to increase momentum (Okeke,1987). It has also been reported (Okeke,1987) that when the momentum of a moving object is increased, the force called into play also increases.

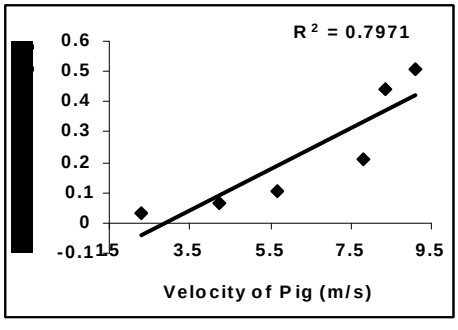

Fig. 1 Effect of velocity on Force of Erasure

The results generated in this field work as shown in Figs. 1 & 2 for PPL 1, Figs. 3 & 4 for PPL 2 and Figs. 5 and 6 for PPL 3 respectively indicate that as the velocity of running Pig is increased during cleaning of pipelines, the force exerted by the moving Pig during this cleaning process also increases resulting in the increased dislodgment and removal of unwanted debris within the pipeline. This invariably enhances the efficiency of fluid flow through such pigged pipelines.

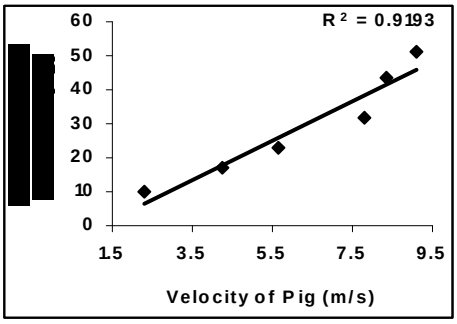

Fig. 2 Effect of velocity on the mass of debris removed

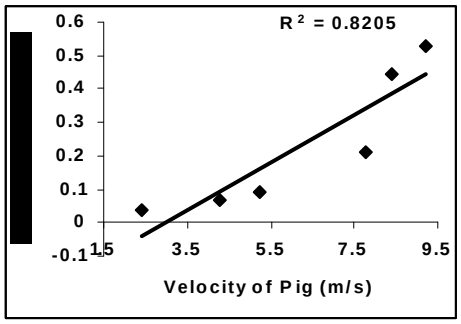

Fig. 3 Effect of velocity on Force of Erasure

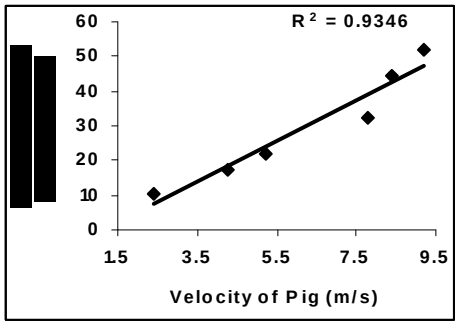

Fig. 4 Effect of velocity on the mass of debris removed

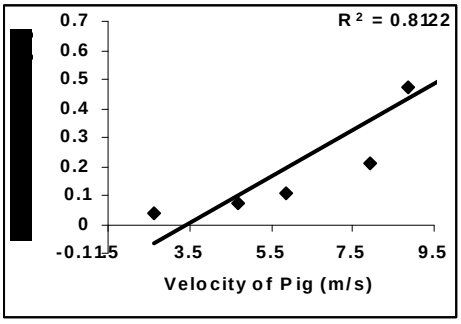

Fig. 5 Effect of velocity on Force of Erasure

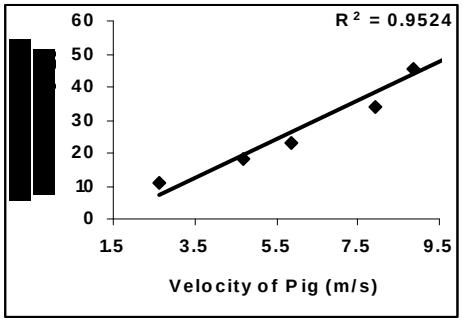

Fig. 6 Effect of velocity on the mass of debris removed

Table 1: Time elapse (seconds) during the pigging process in pipelines

PPL 1	PPL 2	PPL 3
3580	3541	3498
3431	3428	3396
2998	3001	2989
1996	2006	1996
1023	1016	1003
967	942	842

Effect of pigging on the efficiency of fluid delivery through pipeline system

The primary purpose of any pipeline maintenance programme is to maximize flow ability and prolong the life of the pipeline system. To calculate the efficiency of fluid delivery through pipeline system;

Let

α_i = Installed mass flow rate of fluid through the pipeline (i.e when pipeline is devoid of obstacles) (kg/s)

α_o = Obstructed mass flow rate of fluid through the pipeline (kg/s)

In both cases, (installed and obstructed mass flow rates), the mass of fluid flowing through the pipeline is the same. The difference in their flow rates stems on their velocities of flow. The velocity of flow of fluid through the pipelines of obstructed internal diameter was observed to be lower than that obtained for clean and newly installed pipelines functioning at its installed capacity.

Mass flow rate is given by;

$$\alpha = \text{Mass} \times \text{Velocity} \quad (9)$$

Based on the foregoing,

$$\alpha_i > \alpha_o \quad (10)$$

Considering equation (9) and substituting in equation (10)

$$MV_i > MV_o \quad (11)$$

Where

V_i = Installed velocity of fluid flow through the pipeline (m/s)
V_o = Obstructed velocity of fluid flow through the pipeline due to obstacle (m/s)

Generally,

$$\text{Efficiency} = \left(\frac{\text{output}}{\text{input}}\right) \times 100 \quad (12)$$

Therefore, the efficiency of fluid flow through pipeline of known installed capacity is given by;

$$E = \left(\frac{\alpha_o}{\alpha_i}\right) \times 100 \quad (13)$$

Where

α_o = output
α_i = input

Substituting equation (11) from equation (10) into equation (13)

$$E = \left(\frac{MV_o}{MV_i}\right) \times 100 \quad (14)$$

Since the mass of fluid flowing through the pipeline is constant,

$$E = \left(\frac{V_o}{V_i}\right) \times 100 \quad (15)$$

Since $V_i = V_o$, effective pigging and cleaning of the pipeline would have the effect of increasing the efficiency of flow in equation (10) since the value of V_o increases. High efficiency of fluid flow is achieved when the inner part of the pipe is so effectively cleaned that there is very little or no obstacle in form of dirt, corrosion products, wax and particles inside the pipes. In this situation, the value of V_o increases greatly and become very close to V_i, hence making efficiency E very close to 100%. Therefore, pigging pipelines with achievable high degree of cleanliness within the internal diameter, and along the entire length of the pipeline gives very high value of V_o and evaluation of E in this case gives a very high value. Based on this, it clear that when pigging of oil field pipelines are carried out obtaining high degree of cleanliness inside the pipeline, there is high efficiency of fluid delivery to predetermined destination, all other factors being constant.

4. Conclusion

Studies carried out to investigate the effect of velocity of cleaning Pigs on the efficiency of fluid delivery in pigged pipeline system shows that increase in the velocity of the Pig increases the acceleration of the Pig leading to increase in the force exerted on the wall of the pipe. This resulted to increased dislodgment and removal of corrosion products, films, particles, wax and other unwanted debris accumulated within the pipeline. The efficiency of fluid flow through pigged pipelines is dependent on the velocity of fluid flow (after pigging) which in turn depends on the velocity with which the Pig ran during the cleaning process.

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Table 2: Effect of velocity of fluid on its flow efficiency

	PPL 1		PPL 2		PPL 3	
	Bp	Ap	Bp	Ap	Bp	Ap
Velocity (V_o) (m/s)	1.96	2.78	2.01	2.76	1.98	2.75
Efficiency (E) (%)	70	99.29	71.79	98.57	70.71	98.21

Installed velocity (V_i) of fluid flow through the pipeline: 2.8 m/s

Where

Bp = Before pigging
Ap = After pigging

Technical Report

Studies on Inhibition of Microbial Induced Corrosion through Biocide Injection and Determination of Conditions for Assurance of Pipeline Integrity

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Abstract: Studies on the inhibition of microbial induced corrosion through biocide injection and determination of conditions for assurance of pipeline integrity have been carried out. The results of the investigation indicate that biocide requirement, biocide injection rate and pump stroke per minute increase with increase in both the water cut and gross fluid flow. It was found that (based on the convectional conditions Bpd/Br $\geq 17.857 \times 10^3$ and Br/SPM ≥ 0.28) pipeline integrity would be maintained at gross fluid flow range 123036-147857Bpd when the water cut is constant (2%) or at water cut range 1.8-2.0% when the gross fluid flow is constant (135000Bpd). It was also found that based on the formulated condition (Bpd/SPM ≥ 5000) in this work, pipeline integrity is assured at gross fluid flow range 123036-147857Bpd when the water cut is maintained constant at 2% or at water cut range 1.8-2.0% when the gross fluid flow is constant; at 135000Bpd. The short stroke pump was found to give more realistic and accurate biocide injection rate than the long stroke pump. [Report and Opinion. 2009;1(5):19-24]. (ISSN: 1553-9873).

Keywords: Effect, Biocide, Microbial Induced Corrosion, Oil Pipeline Integrity.

1. Introduction

Microbial corrosion, as the name implies, is a kind of corrosion caused or enhanced by micro-organisms, particularly sulphate reducing bacteria, although some other microbes are known to play a secondary role. There are two ways in which micro-organisms are involved in corrosion processes. Firstly, by virtue of their growth and metabolism they can introduce into an innocuous system, chemical entities such acids, alkali, sulphides, and other aggressive ions which will render the environment corrosive. Secondly, their presence could provide the structure with concentration cell, with some areas being anodic compared to the rest (Ijomah,1991).

Biofouling is the deposition onto a metal surface, of particles normally suspended in solution (Crook,1986). The colloidal matter could come from so many sources both internal and external. They could enter the process through make up water, especially the untreated surface water. It could be scrubbed from the air as in open recirculation systems and could also come from organic deterioration products. These particles tend to take up a charge opposite to that of the metal surface, and fouling results causing a decline in heat transfer (Crook,1986). Algae, i.e., chlorophyll-containing organisms that need light for growth, are usually present in industrial cooling towers. If allowed unchecked, they are capable of producing great masses of material whose weight could endanger the structure and whose mass impedes air and water flow. Even small growths could slough off and be carried into circulating stream as fouling matter.

Slimes normally contain fungi, yeast, bacteria and trapped quantities of organic and inorganic matter (Crook,1986). Slimes forming bacteria are usually encapsulated in this gelatinous mass. Alive, they attach themselves to steel surfaces and grow to restrict heat transfer. In extreme cases, they can also restrict water flow and the deposits also set up concentration cells, causing corrosion (Crook,1986).

Aerobic iron bacteria can oxidize ferrous ions in solution to the ferric state and thus effect the precipitation of ferric hydroxide (Loverell,1989). These organisms are common inhabitants of springs and may find their way into water pipes. Precipitated ferric hydroxides can build up on the internal surface of a pipe to form hard excrescences known as tubercles, which are firmly adherent to the metal surface (Booth,1985). The tubercle shields the surface of the pipe from contact with

dissolved oxygen (Booth,1985). Hence, the metal at the base of the tubercle becomes anodic to those parts not covered by the deposit. The problem is often intensified by the fact that sulphate reducing bacteria takes advantage of the anaerobiosis created by the tubercles to make their input to the total corrosion.

Most instances of corrosion in the absence of oxygen have been attributed to the sulphate reducing bacteria (SRB), of genera; disulfovibrio and disulfotomaculum (Nwoye,2002). Typical examples of such an environment are natural water-logged soils and waters heavily polluted with organic matter. These microbes are obligate anaerobes, i.e, they will not grow in the presence of even traces of oxygen, but usually grow well at pH between 5 and 9 and temperatures between 25 and 44^0C, although some strains (disulfotomaculum nigrificans) are able to withstand higher temperatures (Crook,1986). They utilize hydrogen or some reducing substances for their life process. The corrosion is often localized and is generally characterized by a black corrosion product with a strong smell of hydrogen sulphide (H_2S) (Crook,1986). Sulphate reducing bacteria can cause serious damage in buried pipes, central heating installations, heat exchangers and cooling towers.

Mechanism of attack by sulphate reducing bacteria

Sulphate reducing bacteria have been found (Crook,1986) to utilize hydrogen for their metabolim. It then follows that for an iron or steel structure which has become polarized through the formation of hydrogen at the cathode and Fe^{2+} at the anode, the bacteria obtain their necessary hydrogen at the cathodic site, thus depolarizing the cathode. The S^{2-} ions arising from the sulphate reduction process then combines with Fe^{2+} ions to form ferrous sulphide (FeS). Hence, the anode reaction is also polarized, allowing the attack to proceed unhindered. Mathematically, the reaction is believed to follow the scheme (Crook,1986):

$$Fe = Fe^{2+} + 2e \quad \text{(anodic reaction)} \qquad (1)$$

$$2H^{+} + 2e = \quad \text{(cathodic reaction)} \qquad (2)$$

$$SO^{2-}_4 + 8H = S^{2-} + 4H_2O \qquad (3)$$
(cathode depolarization by SRB)

$$Fe^{2+} + S^{2-} = FeS \quad \text{(corrosion product)} \qquad (4)$$

$$FeS + 2H_2O = H_2S + Fe(OH)_2 \qquad (5)$$
(corrosion product)

It was found (Crook,1986) that when microbial growth occurs on a structure liable to corrosion such as pipeline, a differential aeration cell is usually set up between those parts of the structure where oxygen supply has been is depleted and those parts where micro-organisms are not active. The oxygen depleted regions will be anodic to the rest and will therefore become centre for active metal loss.

Prevention of microbial corrosion

The various measures which have been successfully applied to prevent or control microbial corrosion include: cathodic protection, aeration, removal of metabolite and use of inhibitors (Fontana and Greene,1967). Cathodic protection has been used successfully to control microbial corrosion since all the anodic areas are eliminated by making the entire structure the cathode of an electrochemical cell. The cheapest and most effective inhibitor for sulphate reducing bacteria is air or oxygen. Forced aeration of stagnant water has been used to control corrosion in tanks and incidentally, to banish offensive odours while the drainage of waterlogged soils to improve aeration has been used to control the corrosion of buried pipes. Sometimes, it is possible to control microbial action by removal of an essential metabolite from the system. For example, constructing cooling towers to exclude light is an effective means of control against algae. Inhibitors of microbial action are two types. These are biocides which actually kill the organisms and biostats which maintain the organisms in a state of inactivity or non-growth.

When biocides are used to inhibit the microbial actions of the sulphate reducing bacteria, some equations are vital for the control analysis (Okure,2000);

$$(Br) = 0.0028 \times WC \times Bpd \qquad (6)$$

$$(Bj) = Br/t \qquad (7)$$

Substituting the value of Br in equation (6) into equation (Okure,2000)

$$Bj = \frac{0.0028 \times WC \times Bpd}{t} \qquad (8)$$

Where

Br = Biocide requirement (US Galls.)
Bj = Biocide injection rate (US Galls./hr)
Bpd= Gross fluid flow
WC = Water cut (%)
t = Time elapse during the fluid flow (hr)
0.0028 = Flow constant

The aim of the present work is to study the inhibition of microbial induced corrosion through biocide injection and to determine conditions for assurance of pipeline integrity. In this work biocide was used to wipe the pipes conveying oil clean of these sulphate reducing bacteria so as to maintain high efficiency and uninterrupted flow of the fluid through the pipes. This way, it is expected that pipeline integrity would be maintained.

2. Materials and methods

Three major oil pipelines at offshore platforms in Akwa Ibom State, certified infested by sulphate reducing bacteria (following preliminary phynotipic examination carried out) were worked on during this study. Varied values of water cut (WC) and gross fluid flow (BPD) through the line to be treated were considered in order to evaluate the respective associated biocide requirements. The expected biocide requirement was based on 4 hrs fluid flow. Biocide injection rates were calculated and used to evaluate (by interpolation) the pump stroke per minute (both short and long stroke) using values obtained from the usage of Texsteam 5006 chemical injection pump. These values are presented in Table 1. Other results generated in the course of the research work are presented in Tables 2-5. Details of the stages of the experiments and techniques used are as stated in previous report (Nwoye,2000).

3. Results and discussion

Fig.1 shows that at constant gross fluid flow (135000Bpd), biocide requirement increases with increase in the water cut.

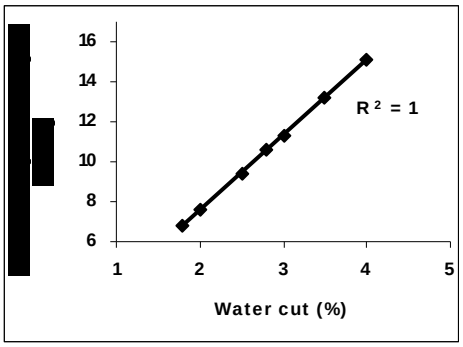

Fig. 1 Variation of water cut with biocide requirement at constant gross fluid flow

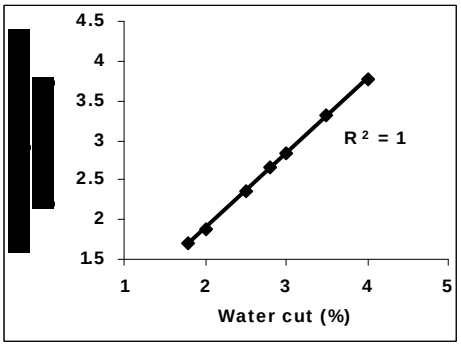

Fig. 2 Variation of water cut with biocide injection rate at constant gross fluid flow

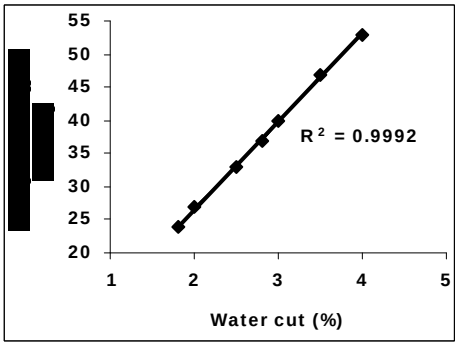

Fig. 3 Variation of water cut with pump stroke per minute obtained from short stroke

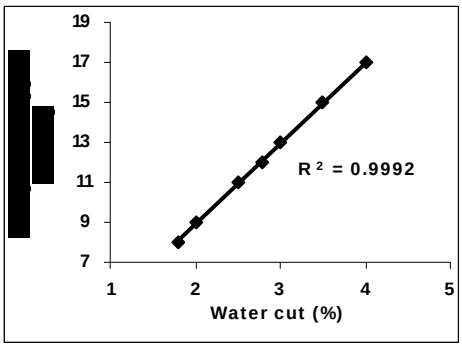

Fig. 4 Variation of water cut with pump stroke per minute obtained from long stroke

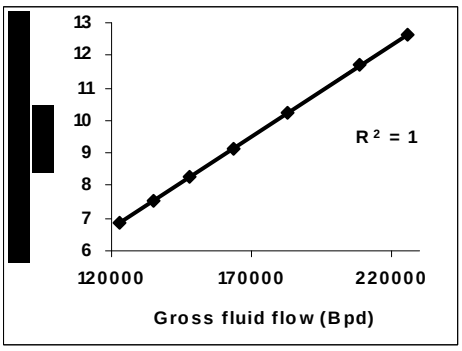

Fig. 5 Variation of gross fluid flow with biocide requirement at constant water cut

This was also the case with biocide injection rate in relation to the water cut (Fig. 2). The pump stroke per minute (SPM) calculated using the long and short pump strokes were also found to increase with increase in the water cut. This is shown in Figs. 3 and 4. Figs. 5 and 6 (at constant water cut of 2%) also indicate that both the biocide requirement and biocide injection rate increase respectively with corresponding increase in the gross fluid flow.

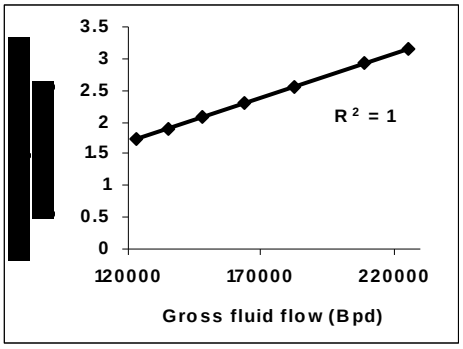

Fig. 6 Variation of gross fluid flow with biocide injection rate at constant water cut

Figs. 7 and 8 also show that the pump stroke per minute evaluated using short stroke and long stroke increase with increase in the gross fluid flow. Comparison of Figs. 3, 4, 7 and 8 shows that greater values and better relationship for pump stroke per minute is obtained as water cut varies and the gross fluid flow remain constant.

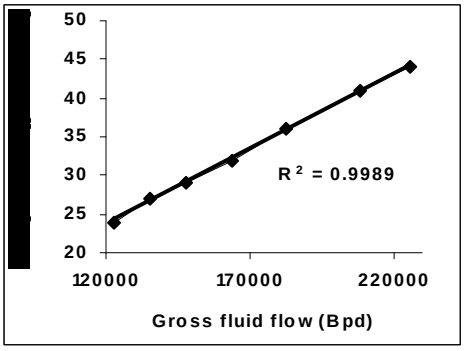

Fig. 7 Variation of gross fluid flow with pump stroke per minute obtained from short stroke

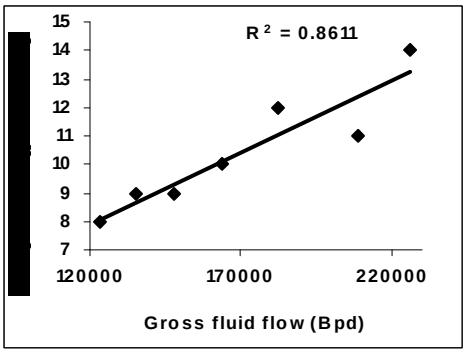

Fig. 8 Variation of gross fluid flow with pump stroke per minute obtained from long stroke

Table 1:Values obtained from Texsteam 5006 chemical injection pump used

SPM	Volume (US Galls./hr)	
	Long Stroke	Short Stroke
5	1.11	0.36
10	2.22	0.71
15	3.33	1.07
20	4.44	1.42
25	5.55	1.78
30	6.66	2.13
35	7.77	2.49
40	8.88	2.84
45	9.99	3.20
50	11.10	3.55
55	12.21	3.91

Table 2: Variation of Bpd/Br and Br/SPM with water cut at constant gross fluid flow

WC (%)	Bpd/Br (10^3)	Br/SPM
2.0	17.857	0.2800
2.5	14.286	0.2864
1.8	19.853	0.2833
3.0	11.905	0.2835
2.8	12.760	0.2859
3.5	10.204	0.2815
4.0	8.929	0.2853

Table 3: Variation of Bpd/Br and Br/SPM with gross fluid flow at constant water cut

Bpd	Bpd/Br (10^3)	Br/SPM
123036	17.857	0.2871
135000	14.286	0.2800
147857	19.853	0.2855
163571	11.905	0.2863
182500	12.760	0.2839
208571	10.204	0.2849
225714	8.929	0.2873

Table 4: Variation of Bpd/SPM with water cut at constant gross fluid flow (for the derived expression)

Bpd/SPM (10^3)	Bpd
5.1268	123036
4.0000	135000
5.6680	147857
3.4083	163571
3.6226	182500
2.9071	208571
2.5652	225714

Table 5: Variation of Bpd/SPM with gross fluid flow at constant water cut (for the derived expression)

Bpd/SPM (10^3)	WC (%)
5.1268	2.0
4.0000	2.5
5.6680	1.8
3.4083	3.0
3.6226	2.8
2.9071	3.5
2.5652	4.0

This is confirmed by the R^2 values from the respective figures which are 0.9992, 0.9992, 0.9989 and 0.8611 respectively. These R^2 values translate into the correlation coefficients R; 0.9996, 0.9996, 0.9994 and 0.9280 respectively following evaluation of the square root of R^2. Comparison of Fig. 7 and 8 shows (at constant water cut (2%)) a better relationship between pump stroke per minute and gross fluid flow as evaluated using short stroke than in the case of long stroke. Their respective correlation coefficients (0.9994 and 0.9280) confirm this. This is a clear indication that usage of short stroke pump gives more accurate biocide injection rate for treatment of SRB infested pipelines compared to long stroke pump.

Determination of conditions for Pipeline integrity

It has been found (Okure,2000) that pipeline maintains its integrity during flow of fluid when the expression;

$$\frac{Bpd}{Br} \geq 17.857 \times 10^3 \quad (US\ Galls.)^{-1} \qquad (9)$$

or

$$\frac{Br}{SPM} \geq 0.28 \qquad (10)$$

Where SPM is the pump stroke per minute.

Multiplying equations (9) by (10)

$$\frac{Bpd}{SPM} \geq 4999.96 \qquad (11)$$

Therefore, based on this derived expression (equation (11)), for pipeline to maintain its integrity,

$$\frac{Bpd}{SPM} \geq 5000 \quad \text{approximately)} \qquad (12)$$

Some results generated in the course of this work shows that at certain conditions, the derived expression (equation (12)) is valid for short stroke pump which has already been determined in this work (comparing Figs.7 and 8) to give more realistic and accurate biocide injection rate than the long stroke pump. Table 2 shows that at constant gross fluid flow, Bpd/Br & Br/SPM values are 19853 & 0.2833 at 1.8% water cut and 17857 & 0.28 at 2% water cut respectively. At constant water cut (2%), Table 3 indicates that Bpd/Br & Br/SPM values are 19853 & 0.2855 at gross fluid flow ; 147857Bpd and 17857 & 0.2871 at gross fluid flow;123036 Bpd respectively. Similarly, results shown in Table 4 indicate that at constant gross fluid flow, the evaluated values of Bpd/SPM = 5668 and 5126.8 at water cut values 1.8 and 2% respectively. Table 5 also shows that at constant water cut, Bpd/SPM values evaluated are 5668 and 5126.8 at gross fluid flow values 123036 and 147857 Bpd respectively.

Based on the foregoing, it is a clear indication that pipeline integrity would be maintained at gross fluid flow range 123036-147857Bpd when the water cut is constant (2%) or at water cut range 1.8-2.0% when the gross fluid flow is constant (135000Bpd). This analysis is based on the convectional conditions (Bpd/Br $\geq 17.857 \times 10^3$ and Br/SPM $\geq$ 0.28) for evaluating pipeline integrity. Based on the condition (Bpd/SPM $\geq$ 5000) formulated in this work, pipeline integrity is ensured at gross fluid flow range 123036-147857Bpd when the water cut is maintained constant at 2% or at water cut range 1.8-2.0% when the gross fluid flow is constant, maintained at 135000Bpd. This is confirmed in Tables 2-5.

4. Conclusion

Studies carried out on the inhibition of microbial induced corrosion through biocide injection indicate that biocide requirement, biocide injection rate and pump stroke per minute increase with increase in both the water cut and gross fluid flow. It was found that (based on the convectional conditions Bpd/Br $\geq 17.857 \times 10^3$ and Br/SPM $\geq$ 0.28) pipeline integrity would be maintained at gross fluid flow range 123036-147857Bpd when the water cut is constant (2%) or at water cut range 1.8-2.0% when the gross fluid flow is constant (135000Bpd). It was also found that based on the formulated condition (Bpd/SPM $\geq$ 5000) in this work, pipeline integrity is assured at gross fluid flow range 123036-147857Bpd when the water cut is maintained constant at 2% or at water cut range 1.8-2.0% when the gross fluid flow is constant, maintained at 135000Bpd. The short stroke pump was found to give more realistic and accurate biocide injection rate than the long stroke pump.

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7/8/2009

Thermodynamic modeling of an irreversible dual cycle: effect of mean piston speed

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Abstract: In this paper, the theory of finite time thermodynamics is used to determine the effect of mean piston speed on performance of an irreversible dual cycle. In the model, the non-linear relation between the specific heat ratio of the working fluid and its temperature, the friction loss computed according to the mean velocity of the piston, the internal irreversibility described by using the compression and expansion efficiencies and the heat transfer loss are considered. The relations between the power output and the compression ratio, between the power output and the thermal efficiency are derived by detailed numerical examples. The results shows that if compression ratio is less than certain value, the power output increases with increasing mean engine speed, while if compression ratio exceeds certain value, the power output first increases and then starts to decrease with increasing mean engine speed. With further increase in compression ratio, the increase of mean piston speed results in decreasing the power output. This paper provides an additional criterion for use in the evaluation of the performance and the suitability of a dual engine. [Report and Opinion. 2009;1(5):25-30]. (ISSN: 1553-9873).

Key words: dual cycle; internal irreversibility; performance optimization

1. Introduction

Traditional thermodynamics is a theory about equilibrium states and about limits on process variables for transformations from one equilibrium state to another. In order to obtain more realistic limits to the performance of real processes, thermodynamics is extended to finite-time thermodynamics to deal with processes which have explicit time or rate dependencies (Andresen et al., 1984; Bejan, 1996; Aragon-Gonzalez et al., 2000). Thus, much work has been performed for the performance analysis and optimization of finite time processes and finite size devices (Aragon-Gonzalez et al., 2006; Chen et al., 2007; Aragon-Gonzalez et al., 2008; Ge et al., 2008a). Wu and Blank (1992) and Blank and Wu (1994) carried out the effect of combustion on the work or power-optimized Otto, Diesel and dual cycles. Chen et al. (1996) and Chen et al. (1998) derived the relations between net work output and efficiency of the Diesel cycles. The relation between net work output and the efficiency as well as the maximum net-work output and the corresponding efficiency for internal-combustion dual cycles are derived in this paper. Sahin et al. (2002a, 2002b) optimized the performance of a new combined power cycle based on power density analysis of the dual cycle and made a comparative performance analysis of an endoreversible dual cycle under a maximum ecological function and maximum power conditions. Parlak et al. (2004) optimized the performance of an irreversible dual cycle: the predicted behavior was corroborated by experimental results. Chen et al. (2004) determined the characteristics of power and efficiency for dual cycle with heat transfers and friction losses. It is found that there are optimal values of the cut-off ratio at which the power output and efficiency attain their maxima. Ust et al. (2005) performed an ecological performance analysis for an irreversible dual cycle by employing the new thermo-ecological criterion as the objective function. They compared the effects of cut-off ratio on performance of the cycle. Al-Sarkhi et al. (2006) investigated the effects of friction, temperature-dependent specific heat of the working fluid and cut-off ratio on the performances of the Diesel-cycle. Parlak and Sahin (2006) defined the internal irreversibility by using entropy production, and analyzed the effect of the internal irreversibility on the performance of irreversible dual cycle. Chen et al. (2006) and Ghatak and Chakraborty (2007) analyzed the effect of variable specific heats and heat transfer loss on the performance of the dual cycle when variable specific heats of working fluid are linear functions of its temperature. Zhao et al. (2007) defined the internal irreversibility by using compression and expansion efficiencies and analyzed the performance of dual cycle. Ge et al. (2008a; 2008b; 2009) analyzed the performance of an air standard Otto, Diesel and dual

cycles. In the irreversible cycle model, the non-linear relation between the specific heat of the working fluid and its temperature, the friction loss computed according to the mean velocity of the piston, the internal irreversibility described by using the compression and expansion efficiencies, and the heat transfer loss are considered.

As can be seen in the relevant literature, the investigation of the effect of mean piston speed on performance of dual cycle does not appear to have been published. Therefore, the objective of this study is to examine the effect of mean piston speedon performance of air standard dual cycle.

1. Thermodynamic analysis

The temperature-entropy diagram of an irreversible dual heat engine is shown in Fig. 1, where T_1, T_{2s}, T_2, T_3, T_4, T_{4s} and T_5 are the temperatures of the working substance in state points 1, 2s, 2, 3, 4, 4s and 5. Process $1\to 2s$ is a reversible adiabatic compression, while process $1\to 2$ is an irreversible adiabatic process that takes into account the internal irreversibility in the real compression process. The heat additions are an isochoric process $2\to 3$ and an isobaric process $3\to 4$. The process $4\to 5s$ is a reversible adiabatic expansion, while $4\to 5$ is an irreversible adiabatic process that takes into account the internal irreversibility in the real expansion process. The heat-removing process is the reversible constant volume $5\to 1$.

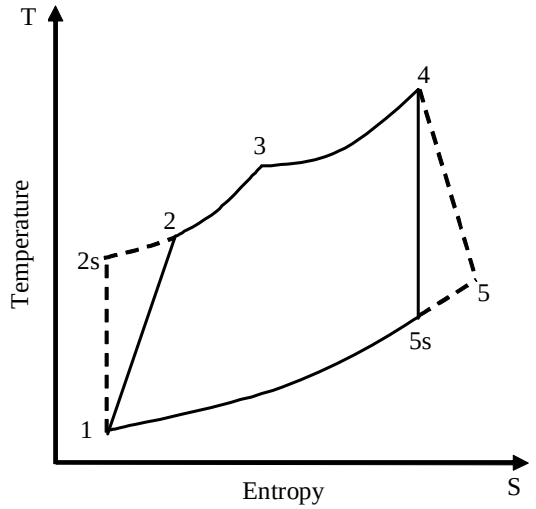

Figure 1. $T-S$ diagram of a dual cycle

According to Refs. (Abu-Nada 2005; Chen et al. 2009), for the temperature range of 300-3500 K, the specific heat with constant pressure can be written as:

$$c_p = 2.506\times10^{-11}T^2 + 1.454\times10^{-7}T^{1.5} - 4.246\times10^{-7}T + 3.162\times10^{-5}T^{0.5} + 1.3303 - 1.512\times10^{4}T^{-1.5} + 3.063\times10^{5}T^{-2} - 2.212\times10^{7}T^{-3} \quad (1)$$

where T is the absolute temperature. The unit of c_p is $kJ\,kg^{-1}K^{-1}$.

The specific heat with constant volume can be written as:

$$c_v = c_p - R_{air} = 2.506\times10^{-11}T^2 + 1.454\times10^{-7}T^{1.5} - 4.246\times10^{-7}T + 3.162\times10^{-5}T^{0.5} + 1.0433 - 1.512\times10^{4}T^{-1.5} + 3.063\times10^{5}T^{-2} - 2.212\times10^{7}T^{-3} \quad (2)$$

The heat added per second in the isobaric ($2\to 3$) and isobaric ($3\to 4$) heat addition processes may be written as

$$Q_{in} = M_{Sp}\left[\int_{T_2}^{T_3} c_v dT + \int_{T_3}^{T_4} c_p dT\right] = M_{Sp}\left[8.353\times10^{-12}T^3 + 5.816\times10^{-8}T^{2.5} - 2.123\times10^{-7}T^2 + 2.108\times10^{-5}T^{1.5} + 1.0433T + 3.024\times10^{4}T^{-0.5} - 3.063\times10^{5}T^{-1} + 1.106\times10^{7}T^{-2}\right]_{T_2}^{T_3} + M_{Sp}\left[8.353\times10^{-12}T^3 + 5.816\times10^{-8}T^{2.5} - 2.123\times10^{-7}T^2 + 2.108\times10^{-5}T^{1.5} + 1.3303T + 3.024\times10^{4}T^{-0.5} - 3.063\times10^{5}T^{-1} + 1.106\times10^{7}T^{-2}\right]_{T_3}^{T_4} \quad (3)$$

where M_{sp} is the molar number of the working fluid which is function of mean engine speed.

The heat rejected per second in the isochoric heat rejection process ($5\to 1$) may be written as:

$$Q_{out} = M_{Sp}\int_{T_1}^{T_5} c_v dT = M_{Sp}\left(8.353\times10^{-12}T^3 + 5.816\times10^{-8}T^{2.5} - 2.123\times10^{-7}T^2 + 2.108\times10^{-5}T^{1.5} + 1.0433T + 3.024\times10^{4}T^{-0.5} - 3.063\times10^{5}T^{-1} + 1.106\times10^{7}T^{-2}\right) \quad (4)$$

The compression and expansion efficiencies can be defined as (Ge et al., 2008a; Ge et al., 2008b):

$$\eta_c = (T_{2s} - T_1)/(T_2 - T_1) \quad (5)$$

and

$$\eta_e = (T_5 - T_4)/(T_{5s} - T_4) \quad (6)$$

These two efficiencies can be used to describe the internal irreversibility of the processes.

Since specific heat with constant volume and specific heat with constant pressure are dependent on temperature, the adiabatic exponent will vary with temperature as well. Therefore, the equation often used in a reversible adiabatic process with constant specific heat ratio cannot be used in a reversible adiabatic process with variable specific heat ratio. However, according to Refs (Ge et al. 2007; Chen et al., 2008), the equation for a reversible adiabatic process with

variable specific heat ratio can be written as follows:

$$TV^{\gamma-1}=(T+dT)(V+dV)^{\gamma-1} \quad (7)$$

From Eq. (6), one gets

$$C_v \ln\frac{T_j}{T_i}=R_{air}\ln\frac{V_i}{V_j} \quad (8)$$

where the temperature in the equation of c_v is $T=\left(T_j-T_i\right)\Big/\ln\left(T_j/T_i\right)$.

The compression ratio, r_c, and pressure ratio, α, are defined as

$$r_c=V_1/V_2 \quad (9)$$

and

$$\alpha=T_3/T_2 \quad (10)$$

Therefore, the equations for processes $(1\rightarrow 2s)$ and $(4\rightarrow 5s)$ are shown, respectively, by the following:

$$C_v \ln\frac{T_{2s}}{T_1}=R_{air}\ln r_c \quad (11)$$

and

$$C_v \ln\frac{T_4}{T_{5s}}=R_{air}\ln\frac{T_2}{T_4}+R_{air}\ln(r_c\alpha) \quad (12)$$

The energy transferred to the working fluid during combustion is given by the following linear relation (Chen et al., 2008; Ebrahimi, 2009b)

$$Q_{leak}=M_{Sp}B\left(T_2+T_4-2T_0\right) \quad (13)$$

where B are a constant related to heat transfer.

Taking into account the friction loss of the piston and assuming a dissipation term represented by a friction force that is a linear function of the piston velocity gives (Chen et al., 2006; Ge et al., 2007; Ebrahimi, 2009a)

$$f_\mu=-\mu S_p=-\mu\frac{dx}{dt} \quad (14)$$

where μ is the coefficient of friction, which takes into account the global losses, x is the piston's displacement and S_p is the piston's velocity. Therefore, the lost power due to friction is

$$P_\mu=\frac{dW_\mu}{dt}=-\mu\left(\frac{dx}{dt}\right)^2=-\mu\left(S_p\right)^2 \quad (15)$$

Thus, the lost power is

$$P_\mu=-\mu\left(\overline{S}_p\right)^2 \quad (16)$$

where $\overline{S}_p$ is the mean velocity of the piston.

Thus, the power output of the dual cycle engine can be written as

$$P_{out}=Q_{in}-Q_{out}-P_\mu \quad (17)$$

The efficiency of the dual cycle engine is expressed by

$$\eta_{th}=\frac{Q_{in}-Q_{out}-P_\mu}{Q_{in}+Q_{leak}}=\frac{P_{out}}{Q_{in}+Q_{leak}} \quad (18)$$

When r_c, α, T_1, T_4, η_c and η_e are given, T_{2s} can be obtained from Eq. (11), then, substituting T_{2s} into Eq. (5) yields T_2. T_3 can be obtained from Eq. (10), T_{5s} can be obtained from Eq. (12) and the last, T_5 can be found by substituting T_{5s} into Eq. (6). Substituting T_1, T_2, T_3, T_4 and T_5 into Eqs. (17) and (18), respectively, the power output and thermal efficiency of the dual cycle engine can be obtained. Therefore, the relations between the power output, the thermal efficiency and the compression ratio can be derived.

3. Results and discussion

The following constants and parameters have been used in this exercise: $T_4=2200\,K$, $T_1=350\,K$, $L=70\ mm$, $\eta_c=0.97$, $\eta_e=0.97$, $\mu=12.9\,Nsm^{-1}$, $B=0.2\ kJ.kg^{-1}K^{-1}$, $\overline{S}_p=7\rightarrow 23\ rev/s$, $r_c=1.5\rightarrow 100$, $\alpha=1.5$ $T_0=345\,K$ and $M_{Sp}=5.4204E-4\times\overline{S}_P\ kg.s^{-1}$ (Heywood, 1988; Chen et al. 2007; Ghatak and Chakraborty, 2007; Ge et al., 2009; Ebrahimi, 2009a). Using the above constants and range of parameters, the power output versus compression ratio characteristic and the power output versus efficiency characteristic with varying the mean piston speed can be plotted. Numerical examples are shown as follows.

Figures 2 and 3 show the effects of the variable mean piston speed on the cycle performance with heat resistance, internal irreversibility and friction losses (the dashed lines in the figures denote where the cycle cannot work normally). From these figures, it can be found that the mean piston speed plays important roles on the power output. It is clearly seen that the effect of mean piston speed on the power output is related to compression ratio. They reflect the performance characteristics of a real irreversible dual cycle engine. It should be noted that the heat added and the heat rejected by the working fluid increase with increasing mean piston speed (see Eqs. (3) and (4)).

Figure 2 indicates the effects of the mean piston speed on the power output of the cycle for different values of the compression ratio. It can be seen that the power output versus compression ratio characteristic is approximately parabolic like curves. In other word, the power output increases with increasing compression ratio, reach their maximum values and then decreases with further increase in compression ratio. The maximum power output increases with increasing mean piston speed up to about 15 rev/s where it reaches its peak value then starts to decline as the mean

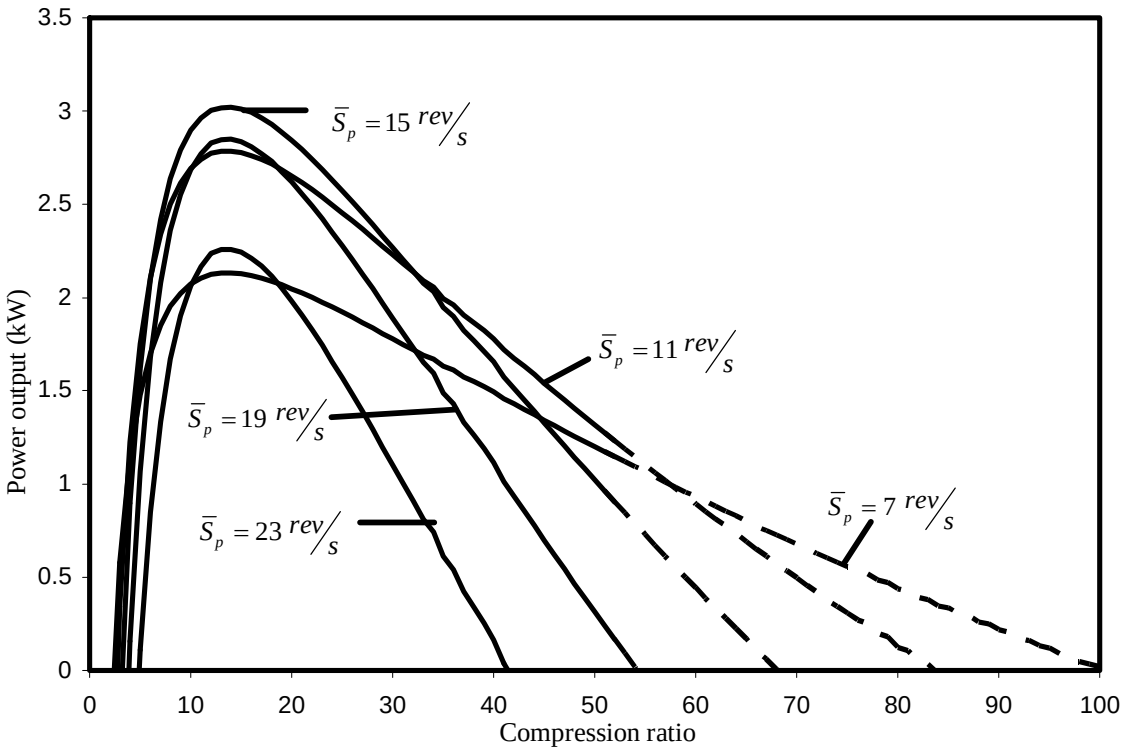

Figure 2. Effect of mean piston speed on the variation of the efficiency with compression ratio

piston speed increases. This is consistent with the experimental results in the internal combustion engine (Mercier, 2006).

The optimal compression ratio corresponding to maximum power output point remains constant with increase of mean engine speed. The results shows that if compression ratio is less than certain value, the power output increases with increasing mean engine speed, while if compression ratio exceeds certain value, the power output first increases and then starts to decrease with increasing mean engine speed. With further increase in compression ratio, the increase of mean piston speed results in decreasing the power output. Numerical calculation shows that for any same compression ratio, the smallest power output is for $\bar{S}_p = 23\ rev/s$ when $r_c \le 10$ or $r_c > 19$ and is for $\bar{S}_p = 7\ rev/s$ when $10 < r_c \le 19$ and also the largest power output is for $\bar{S}_p = 7\ rev/s$ when $r_c \le 3.8$ or $r_c > 57$, is for $\bar{S}_p = 11\ rev/s$ when $3.8 < r_c \le 6$ or $32 \le r_c \le 57$ and is for $\bar{S}_p = 15\ rev/s$ when $6 \le r_c < 32$.

The influence of the mean piston speed on the power output versus thermal efficiency is displayed in figure 3. As can be seen from this figure, the power output versus thermal efficiency is loop shaped one. It can be seen that the power output at maximum thermal efficiency improves with increasing mean piston speed from 7 to around $\bar{S}_p = 15\ rev/s$. With further increase in mean engine speed, the power output at maximum thermal efficiency decreases. It can also be seen that the thermal efficiency at maximum power decreases with increase of mean piston speed from 7 to $\bar{S}_p = 23\ rev/s$.

According to above analysis, it can be found that the effects of the mean piston speed on the cycle performance are obvious, and they should be considered in practice cycle analysis in order to make the cycle model be more close to practice.

4. Conclusion

In this paper, an irreversible air standard dual cycle model which is closer to practice is established. The relations between net power output, efficiency, compression ratio, and the mean piston speed are derived. The maximum power output and the corresponding efficiency and the maximum efficiency and the corresponding power output are also calculated. The detailed effect analyses are shown by numerical examples. The analysis helps us to understand the strong effect of mean piston speed on the performance

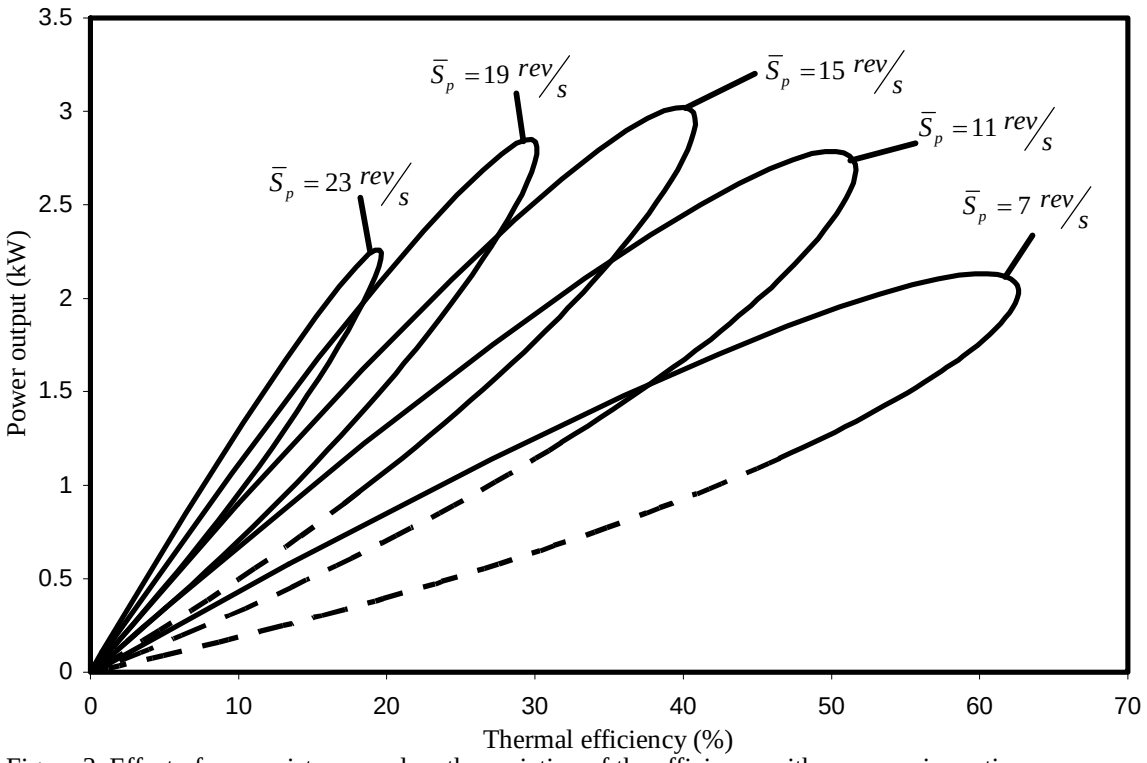

Figure 3. Effect of mean piston speed on the variation of the efficiency with compression ratio

of the dual cycle. This paper provides an additional criterion for use in the evaluation of the performance and the suitability of a dual engine.

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8/27/2009

The Water Chemistry, Phytoplankton Biomass (Chlorophyll *a*), Episammic and Periphytic Algae of the Apese Lagoon, Lagos.

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ABSTRACT: The Apẹsẹ lagoon, hitherto is unreported in literature. The water chemistry, phytoplankton biomass (chlorophyll *a*), episammic and periphytic algae of the Apẹsẹ lagoon and the proximate sea in Lagos were investigated. Samples were collected in Feb., 2009 as part of a series of studies on the ecology of the Apẹsẹ lagoon. Preliminary results from the study revealed a classical tropical marine lagoon. Records show alkaline pH, high brackish conditions, salinity, conductivity and total dissolved solids comparative to a good number of lagoons in the region. Furthermore high dissolved oxygen and low biological oxygen demand were also associated with the lagoon. Comparatively, the marine conditions recorded for the Apẹsẹ lagoon were below records from the simultaneously investigated proximate sea with regard to marine conditions. A total of 34 algal species from 26 genera were recorded. Diatoms (25 species) and blue-green algae (8 species) were the more important groups occurring with regard to diversity and density of occurrence. In terms of composition and abundance, the periphytic community was richer (23 taxa) than the episammic assemblage (15 taxa). Chlorophyll *a* levels were higher in the lagoon than the sea. However, levels were lower with regard to reports for the region. The chlorophyll *a*, water chemistry and microalgal components reflected a tropical unpolluted marine aquatic environment. Relationship to other lagoons in the region is also highlighted within. There is need for further hydrological and ecological studies of the Apẹsẹ lagoon. [Report and Opinion. 2009;1(5):31-40]. (ISSN: 1553-9873).

Key words: water chemistry, phytoplankton biomass, episammic, epiphytic, Apẹsẹ lagoon, south-western Nigeria.

1. INTRODUCTION

Lagoons are prominent hydrological features along the West African Coast (Onyema *et al*., 2008). They are ecologically and economically important aquatic ecosystems in South-western Nigeria. Additionally, they are important for food especially fish, in water transportation, energy generation, exploitation and exploration of some mineral resources including sand (Kirk and Lauder, 2000; Onyema *et al*., 2007; Chukwu and Nwankwo, 2004; Onyema and Nwankwo, 2009).

The fundamental importance of algal components in trophic relationships in the aquatic environments as autotrophs and their bio-indicator value have been assessed and reported in literature over the years (Palmer, 1969; Valandingham, 1989, Dakshini and Soni, 1982). Similarly, in South-western Nigeria there are reports on some of the lagoons and adjoining creeks to this regard (Onyema and Nwankwo, 2006; Onyema, 2007b; Nwankwo and Akinsoji, 1989, 1992; Nwankwo, 2004b).

Microlgal components satisfy conditions to qualify as suitable indicators in that they are simple, capable of quantifying changes in water quality, applicable over large geographic areas and can also furnish data on background conditions and natural variability (Onyema, 2007b). More so micro-algal components respond rapidly to perturbations and are suitable bio-indicators of water conditions which are beyond the tolerance of many other biota used for monitoring (Nwankwo and Akinsoji, 1992; Nwankwo, 2004; Onyema, 2007b). Some related investigations on attached algal components include Nwankwo *et al*., (1994), Onyema (2007a) and Onyema and Nwankwo (2006).

This is an attempt to report preliminary findings on the Apẹsẹ lagoon which has previously remained unreported. This account hence gives first time records for physico-chemical characteristics and aspects of the micro-algal component of the lagoon.

2. MATERIALS AND METHODS

Probable Mode of Formation of the Apese Lagoon from the Former Kuramo Lagoon.

Before now, there were 9 reported lagoons in the south-western Nigeria (Onyema, 2008). The discovery of an additional lagoon (Apẹsẹ lagoon) in

the region was made possible using the Google earth satellite mapping software. This was before *in situ* confirmation and pilot studies were carried out for validation. It is possible that the Apęsę lagoon was 'created' out of the previously existing Kuramo lagoon (Hill and Webb, 1958; Sandison, 1966; Sandison and Hill, 1966). In previous reports, the Kuramo lagoon was much longer and extended further to the east of its current coverage. Coastal sediment build up may have filled up the median portion of the former Kuramo lagoon giving rise to a dichotomy i.e. Kuramo lagoon to the West and Apęsę lagoon to the east. It is additionally possible that the exacerbated erosion effect from the construction of the west and east moles (1901 to 1930) had ebbed and possible accretion/build up of sediment followed at and around the east sea shore of the Kuramo lagoon. This probably elicited the fill up of part of the lagoons length. Consequently, giving rise to two independent and distinct lagoons. Reports from the inhabitants suggest that the Apese lagoon was formerly joined to the present Kuramo lagoon in the past.

Description of the Study Lagoon

The Apęsę lagoon is the smallest of the ten lagoons in South-western Nigeria (Fig 1). It lies between Latitude 6° 25"20.8N, Longitude 3° 27"15.5E and Latitude 6° 25" 20.29N, Longitude 3° 27"57.1E (Onyema, 2009). The region is located in Victoria Island, Lagos state. The lagoon is lanceolate in shape and approximately 32,000m^2 in coverage area, 1.3km long and 0.16km across its widest extreme. It is located directly eastward of the Kuramo lagoon about 1.24km. The lagoon is about one third of the coverage of the Kuramo lagoon. The lagoon is also separated from the proximate sea by less than 100m of sand bar (beach).

The area is exposed to the wet (May - November) and dry season (December to April) with the wet season having a bimodal distribution. Reports from the locals suggest that in the wet season the water volume of the lagoon increases in volume and the depth increases. Parts of the littoral zone are submerged. The vegetation type of the Apęsę lagoon shore is a strand of scrubby vegetation similar to that described by Akinsoji *et al*., (2002) for the shore of the Light house beach. Some species occurring at the Apęsę lagoon include *Ipomoea pes-caprae*, *Philoxerus* sp., *Paspalum vaginatum, Schizachryrium pulchellum* and *Remirea maritima*. Artisanal fishing and the collection of molluscan shells as souvenir for tourist in the lagoon and the sea is the main occupation of the few inhabitants of the immediate area.

It is worthy of note that a good number of the exactitudes for the Apęsę lagoon were obtained by using the Google earth satellite mapping software.

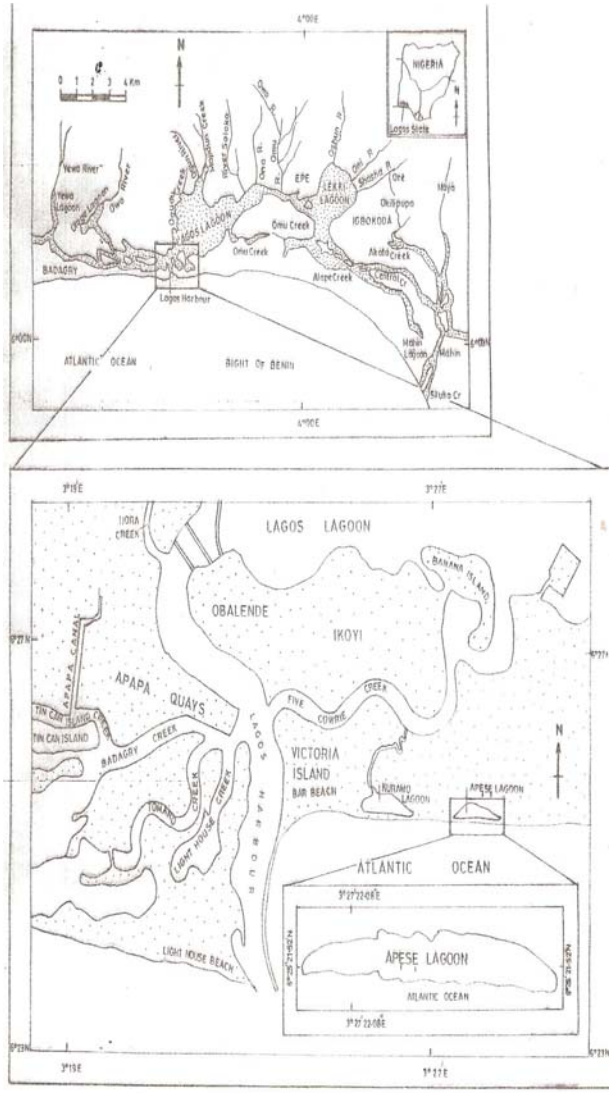

Fig 1: South-western Nigeria lagoons and a map trail to Apesę lagoon (bottom left).

Water sample collection:

Surface water samples were collected from the lagoon at stations A (Latitude 6° 25' 20.32" N, Longitude 3° 27"27.61E) and B (Latitude 6°

27''19.20N, Longitude 3° 27'' 44.51E) and at the nearby sea (Atlantic ocean) (Station C - Latitude 6° 25''16.11N, Longitude 3° 27''35.12E) about 80m away (across the beach berm). The two samples for the lagoon were collected just a few centimeters below the water surface for physical and chemical characteristics analysis using 500ml plastic containers with screw caps at noon on the 2nd of February, 2009 at the Apęsę lagoon. At the ocean front (Station C), water sample was collected by moving inward about 3m from the mid splash point zone. The plastic containers for both the lagoon and ocean samples were then labeled appropriately and transported to the laboratory and subjected to immediate physical and chemical characteristics analysis.

Collection of episammic and periphytic algal samples.

Sediment (episammic) samples were collected from exposed intertidal zone at low tide and at stations A and B. Sediment samples that were suspected to contain algal components as a result of their greenish colouration were collected. In collection, methods described by Hendey (1964) and Onyema (2007a) were employed. Consequently a 5cm^2 strip of the exposed inter – tidal zone at low tide was scrapped with a spatula into two appropriately labeled petri dishes (Onyema, 2007a). The samples were then slightly wetted with 4% unbuffered formalin for preservation and labeled before onward transportation to the laboratory.

In the collection of periphytic algal samples, plastic container found with recognizable growth of algae (i.e greenish / slimy appearance) at the lagoon shores at stations A and B were recovered and dabbed with formalin till all the greenish growth had been soaked. The recovered plastic containers were then put in a black polyethylene bag that was labeled aptly to reflect collection details for both stations. The samples were then preserved with 4% unbuffered formalin.

Analysis of sediment (episammic) and periphytic algal samples.

In the laboratory, the sediment was mixed with distilled water, slackened thoroughly and washed to facilitate the dislodgement and extraction of the algae as described by Hendey (1964). The supernatant thereof was then concentrated to 20ml prior to microscopic analysis as described by Onyema and Ojo (2008). Light microscope investigations on composition and abundance were conducted within 4hours after which samples were persevered with formalin to enable further investigation and confirmation. The periphytic samples on plastic containers were first of all scrapped with a knife into an empty glass beaker and then further brushed to remove any remaining attached algal components. 20ml of distilled water was then added with formalin for preservation. The constituted sample was then subjected to microscopic analysis as described by Onyema and Ojo (2008) after strong perturbation to allow for proper homogenization. Identification was made using relevant texts (Hendey 1958, 1964; Wimpenny, 1966; Patrick and Reimer, 1966, 1975; Whitford and Schmacher, 1973; Vanlandingham, 1982; Nwankwo, 1990, 1995, 2004; Bettrons and Castrejon, 1999; Lange-Bertalot, 2001; Witkowski *et al*., 2000; Siver, 2003; Rosowski, 2003).

3. RESULTS.

Temperature, water chemistry characteristics and chlorophyll *a* concentration.

Table 1 presents the status of the physico-chemical parameters at the two stations (St. A and St. B) within the Apęsę lagoon in Feb., 2009, their mean, standard error, variance, standard deviation and that of the proximate sea (St. C). Air temperature values were between 31.8 and 32.0 ^{0}C with a mean value of 31.90 ^{0}C for the lagoon whereas the sea recorded 31.9 ^{0}C. Water temperature values were between 28.8 and 28.9^{0}C with a mean value of 28.85 ^{0}C for the lagoon while the sea recorded 27.5 ^{0}C. pH at 26^0 values were also between 8.27 and 8.26 for the lagoon whereas the sea recorded 8.52. Furthermore, conductivity values were between 30610 and 30650 µS/cm respectfully and an average of 30630.00µS/cm at stations A and B, while station C recorded 52150 µS/cm. For Total Suspended Solids, values were between 30 and 31 mg/L for the lagoon whereas the proximate sea recorded 28 mg/L. Total Dissolved solids values recorded a mean of 18023.50 mg/L for the lagoon whereas the sea recorded 31605 mg/L.

Salinity estimates for stations A and B were between 17.1 and 17.3‰ with an average of 17.20‰, whereas the sea recorded 30.8‰. Acidity on the other hand were between 4.5 and 4.48 mg/L with a mean value of 4.49mg/L for the lagoon whereas station C

recorded 4.4 mg/L. Similarly, Alkalinity values were between 280.0 and 281.5 mg/L at stations A and B, whereas station C recorded 350.0 mg/L. Total Hardness were between 21000.0 and 21100 mg/L with a mean value of 21050.00 mg/L for the lagoon whereas the sea recorded 33750 mg/L.

For cations, Calcium recorded lower values than Magnesium and was 1200 mg/L at both stations A and B and the sea recorded 2400 mg/L and Magnesium was between 4306.2 and 4310 mg/L with a mean value of 4308.10mg/L for the lagoon whereas the sea recorded 6638.8 mg/L. For heavy metals, Zinc values were between 0.015 and 0.016 mg/L the lagoon, whereas the ocean front recorded 0.016mg/L. Iron levels was 0.15 for both stations A and B and the sea recorded 0.16 mg/L. Copper values was 0.007 mg/L for both stations A and B, whereas the sea recorded 0.006 mg/L. Chlorides values were between 7623.4 and 7631.3 mg/L with a mean value of 7627.35 mg/L for the lagoon whereas the sea recorded 8022.1mg/L.

For the nutrients, Nitrate values were between 4.1 and 4.2 mg/L with a mean value of 4.15 mg/L at stations A and B, whereas station C recorded 5.1 mg/L. Sulphate values were between 3620.1 and 3625.7 mg/L with a mean value of 3622.90 mg/L for the lagoon whereas the sea recorded 3633.2 mg/L. Phosphate values were between 0.05 and 0.06mg/L with a mean value of 0.055 mg/L at stations A and B, whereas station C recorded 0.06 mg/L. Silica values on the other hand were between 3.6 and 3.7 mg/L with an average value of 3.65 mg/L for the lagoon whereas the sea recorded 3.4 mg/L.

Biological Oxygen Demand at both stations in the lagoon and the sea recorded 2 mg/L. Chemical Oxygen Demand estimates were also between 10 and 11mg/L with a mean value of 10.5 mg/L for the lagoon whereas the sea recorded 12 mg/L. Dissolved Oxygen values were between 4.80 and 4.95mg/L for the lagoon whereas the sea recorded 5.0 mg/L.

Phytoplankton biomass represented by chlorophyll *a* (µg/L) on the other hand was between 6.00 and 7.50 µg/L with a mean value of 6.75 µg/L at stations A and B, whereas the sea recorded 5 µg/L.

Episammic and periphytic algae.

Table 2 present the taxonomy and distribution of microalgal components recorded on the sediment and plastics of the Apẹsẹ lagoon in February, 2009. The episammic and periphytic algae are reported. In Table 2, the episammic / sediment algae are represented by Sediment algae St. A and Sediment algae St. B, whereas the periphytic algae are reported under Periphytic algae St. A and Periphytic algae St. B. A total of 34 microalgal components from 26 genera were identified and recorded.

Episammic algae

The total number of species recorded in station A were 12 while the total number in station B was 13. *Lynbgya limnetica* Lemm. was the more important algal species in terms of number of occurrence (Station B). Stations B recorded some species which were not present at station A (*Actinoptychus splendens* Ehrenberg, *Arachnoidiscus* sp, *Cyclotella* sp., *Paralia sulcata* (diatoms), *Microcystis aureginosa* (blue-green algae). Similarly Station A recorded *Cocconeis placentula* (Ehrenberg), *Gyrosigma scalproides*, *Pleurosigma angulatum*, *Pleurosigma elongatum* (diatoms), *Merismopedia gluca* (blue-green) and *Euglena* sp. (euglenoid) not recorded in Station B.

Periphytic algae

The total number of species recorded in station A were 22 while the total number in station B was 21. *Amphora ovalis*, *Cocconeis placentula* and *Licmophora lyngbei* were more important in terms of number. Stations A and B comprised similar periphytic assemblages. However, *Melosira nummuloides* and *Paralia sulcata* were only recorded in station A while *Pleurosigma angulatum*, *Pleurosigma elongatum*, *Thalasiothrix fraunfeldii* (diatoms) and *Lynbgya martensiana* (blue-green algae) were recorded in higher numbers in station A than in station B. Similarly, in station B *Amphiphora alata*, *Cocconeis discuslus*, *Diploneis crabro* and *Surirella ovata* recoded higher numbers than in station A.

4. DISCUSSION

It's worthy of note that the data from this investigation is preliminary and represent findings at the time of collection (Feb., 2009) which may not necessarily be a true reflection of the seasonality to

which the lagoon is more truly exposed to. Air and water temperatures were within tropical limits for the lagoon and sea. Air temperature was higher than water temperature for the lagoon. According to Onyema (2008) the air is known to heat up faster (from insolation) than the water during the day (in the region) as the sun rises. Consequently, at night also the air usually cools faster than the water.

For all the parameters measured, there were modicum differences between the two sites in the lagoon. However, comparative to the sea, more quantitative disparities were recorded. For instance with regard to salinity, the Apẹsẹ lagoon recorded high brackish water situation. Estimates for pH, conductivity, total dissolved solids, alkalinity, total hardness, calcium, magnesium, iron, chlorides, nitrate, sulphate, phosphate, chemical oxygen demand and dissolved oxygen were higher in seawater than the lagoon water. Conversely, estimates for water temperature, total suspended solids, acidity, zinc, copper, silica and chlorophyll *a* were higher in the lagoon than the sea. pH was alkaline for both the lagoon and sea. Other workers have reported alkaline pH in some lagoons in the area (Onyema *et al*., 2003, 2008; Nwankwo *et al.*, 2008) and linked it to the buffering effect of sea water and associated high levels of dissolved bi-carbonates therein. This condition from the sea may be the strong determinant of the pH of the lagoon. Other lagoons in the region have recorded less alkaline and sometimes acidic conditions (Nwankwo, 1998a,b; Onyema and Nwankwo, 2009; Onyema and Emmanuel, 2009; Nwankwo *et al*., 2008). The direct and strong relationships between salinity, chlorides, conductivity, total hardness and total dissolved solids have been reported by a number of ecologists for the region (Nwankwo 1993, Onyema, 2008; Onyema and Nwankwo, 2009). Similarly, the cations, calcium and magnesium are also known to follow like trend (Onyema and Nwankwo, 2009).

With regard to salinity, cations and associated aforementioned parameters, levels for the sea were higher than estimates for the lagoon. Typically, values were generally about half of estimated levels for the sea at the time. Additionally, values for these parameters for the Apese lagoon were higher than for other lagoons in the region (Nwankwo *et al*., 2003, 2008, Onyema, 2008, Nwankwo, 1998b). Heavy metal levels (copper, iron and zinc) were low for the study. Similarly biological and chemical oxygen demand levels were low. Presently, this may be the lowest for any of the other nine lagoons and manifold creeks in the region. This may additionally point to an unpolluted status for the lagoon. According to Hynes (1960) BOD_5 values higher than $8mgl^{-1}$ points to severe pollution. The creeks and lagoons of south-western Nigeria, apart from their more ecological and economic significance, serve as sink for the disposal of an increasing array of waste types (Onyema, 2007b). Succinct examples of lagoons as sinks include the Lagos, kuramo and Ologe lagoons (Nwankwo, 2004b).

It is important to note that dissolved oxygen values were also comparatively high. The sea estimates for dissolved oxygen were higher though, probably a reflection of the perturbations and agitations of breaking waves at the beach. Further to this, comparative to lagoons in the region (Nwankwo, 1993, 1998a, Onyema and Nwankwo 2009, Onyema *et al*., 2007, Nwankwo *et al*., 2008) higher dissolved oxygen were recorded for this study. Reported nutrient levels (Nitrate, Phosphate, Sulphate and Silica) were within ranges that have been reported for other lagoons. Nutrient levels in the other lagoon are keyed to rainfall influx which introduces a lot of nutrient rich components (allocthonus materials) from land based sources. According to Onyema *et al*., (2003) the diluting and enriching effects of floodwaters, inflow of seawater and the existence of environmental gradients govern the distribution of Lagos lagoon biota. This area (Onyema, 2007a) is also known to maintain high brackish to sea conditions throughout the year. Available chlorophyll *a* concentrations were lower when comparated to reports from the region. Whereas Onyema and Nwankwo (2009) reported a range of 4.2 to 55 µg/L for a two year study of the Iyagbe lagoon, Onyema and Ojo (2008) reported a range of 8.3 to 22.1 µg/L for two stations in the Agboyi creek adjoining the Lagos lagoon over a six month period. According to a chlorophyll *a* scale documented by Suzuki *et al*., (2002) for trophic levels, the Apese lagoon falls into the Oligotrophic category with regard to primary production levels.

It is possible to deduce that from the trend of

comparative data elicited from this preliminary survey, that the sea conditions are largely impacting the ecological characteristics of the Apese lagoon. However there is need for a larger pool of consistent and continuous data to further substantiate this position or otherwise.

Comparatively, the periphytic assemblage was richer (in terms of diversity and abundance) than the episammic community. The algae *Lynbgya limnetica* Lemm was notable in terms of number and was present at both sites. Its important to note that many species reported for this study and community are similar to that reported by Onyema (2007a) for a mudflat (algae) at Tarkwa-bay. This similarity may be keyed to the proximity of the two points (Apese lagoon and bay) to the sea and like sediment characteristics. Pennate diatoms were clearly more diverse (19 species) than any other group. According to Round (1953) and Nwankwo and Akinsoji (1989) the possession of a raphe in diatoms is an asset effective in maneuvering through sediment and hence enhances survival in such habitats. The centric diatoms recorded a total of 6 species. Whereas the periphytic algae recorded a total of 22 species in station A and 21 species in station B, the episammic algae on the other hand recorded 12 species in station A and 13 species in station B. Hence, the plastics were better substrates in terms of diversity and abundance of associated species algal materials.

In general marine conditions were clearly evident in the Apẹsẹ lagoon especially pertaining to its water chemistry characteristics and microalgal components. Species such as *Actinoptychus splendens*, *Melosira moniliformis, Melosira nummuloides, Paralia sulcata, Achnanthes longipes*, *Amphiphora alata, Bacillaria paxillifer*, *Diploneis crabro, Gyrosigma scalproides, Pleurosigma angulatum, Licmophora lyngbei, Licmophora* sp. *Thalasiothrix fraunfeldii, Thalasionema longissima, Merismopedia gluca* are known to reflect alkaline pH and brackish water situations in the region (Nwankwo and Gaya, 1996; Nwankwo and Akinsoji, 1992; Onyema *et al*., 2003, 2007, 2008; Onyema, 2008). The algal diagnosis of the Apese lagoon points to an unpolluted marine lagoon with distinct oligotrophic characteristics. There is hence need for its conservation, because of its unique state and type comparative to lagoons in the region.

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Table 1: Status of Physical and Chemical Parameters of the Apese lagoon at Two Stations (Stations A and B) and the adjacent ocean (Station C) (Feb., 2009).

	PARAMETERS	ST. A	ST. B	Mean	Stand. dev	Variance	Stand. error	ST. C
1.	Air Temperature(^{0}C)	31.8	32.0	31.90	0.141	0.02	0.1	31.9
2.	Water Temperature (^{0}C)	28.9	28.8	28.85	0.071	0.005	0.05	27.5
3	pH at 26^0	8.27	8.26	8.27	0.007	0.00	0.005	8.52
4	Conductivity (µS/cm)	30610	30650	30630.00	28.284	800	20	52150
5.	Total Suspended Solids (mg/L)	30	31	30.50	0.707	0.5	0.5	28
6.	Total Dissolved solids (mg/L)	18020	18027	18023.50	4.949	24.5	3.5	31605
7.	Salinity (‰)	17.1	17.3	17.20	0.141	0.02	0.1	30.8
8.	Acidity (mg/L)	4.5	4.48	4.49	0.014	0.0002	0.01	4.4
9.	Alkalinity (mg/L)	280.0	281.5	280.75	1.061	1.125	0.75	350.0
10.	Total Hardness (mg/L)	21000.0	21100	21050.00	70.711	5000	50	33750
11.	Calcium (mg/L)	1200	1200	1200.00	0.00	0.00	0.00	2400
12.	Magnesium (mg/L)	4306.2	4310	4308.10	2.687	7.22	1.9	6638.8
13.	Zinc (mg/L)	0.016	0.015	0.02	0.00	0.00	0.00	0.016
14.	Iron (mg/L)	0.15	0.015	0.08	0.092	0.008	0.065	0.16
15.	Copper (mg/L)	0.007	0.007	0.01	0.00	0.00	0.00	0.006

16.	Chlorides (mg/L)	7623.4	7631.3	7627.35	5.586	31.205	3.95	8022.1
17.	Nitrate (mg/L)	4.2	4.1	4.15	0.070	0.005	0.05	5.1
18.	Sulphate (mg/L)	3620.1	3625.7	3622.90	3.959	15.68	2.8	3633.2
19.	Phosphate (mg/L)	0.05	0.06	0.055	0.007	0.00	0.005	0.06
20.	Silica (mg/L)	3.6	3.7	3.65	0.071	0.005	0.05	3.4
21.	Biological Oxygen Demand(mg/L)	2	2	2	0.00	0.00	0.00	2
22.	Chemical Oxygen Demand (mg/L)	10	11	10.5	0.707	0.5	0.5	12
23.	Dissolved Oxygen (mg/L)	4.80	4.95	4.875	0.106	0.011	0.075	5.0
24.	Chlorophyll *a* (μg/L)	6.00	7.50	6.75	1.060	1.125	0.75	5

Table 2: Taxonomy and distribution of episammic and periphytic algae at stations A and B in the Apęsę lagoon.

Algal Taxa	Sediment algae St. A	Sediment algae St. B	Periphytic algae St. A	Periphytic algae St. B
DIVISION – BACILLARIOPHYTA				
CLASS - BACILLARIOPHYCEAE				
ORDER I – CENTRALES				
Actinoptychus splendens Ehrenberg	-	*	-	-
Melosira moniliformis Agardh	-	-	*	**
Melosira nummuloides Agardh	-	-	*	-
Arachnoidiscus sp	-	*	-	-
Cyclotella sp.	-	*	-	-
Paralia sulcata Ehrenberg	-	*	*	-
ORDER II – PENNALES				
Achnanthes longipes Agardh	-	-	*	*
Amphiphora alata Eherenberg	-	-	*	**
Amphora ovalis Kutzing	-	-	**	**
Bacillaria paxillifer (O.F. Muller) Hendey	*	*	-	-
Cocconeis discuslus (Schum) Cleve	-	-	*	**
Cocconeis placentula (Ehrenberg)	*	-	**	**
Cymbella affinis Kutzing	*	*	*	*
Diploneis crabro Eherenberg	-	-	*	**
Diploneis sp.	-	*	-	-
Gyrosigma scalproides (Rabh) Cleve	*	-	-	-
Pleurosigma angulatum (Quekett) Wm Smith	*	-	**	*
Pleurosigma elongatum Wm Smith	*	-	**	*
Licmophora lyngbei (Kutzing) Grunow	-	-	**	**
Licmophora sp.	*	*	-	-
Surirella ovata Kutzing	-	-	*	**
Synedra crystallina (Ag) Kutzing	-	-	*	*
Synedra sp.	-	-	*	*
Thalasiothrix fraunfeldii Cleve & Grunow	-	-	**	*
Thalasionema longissima Cleve & Grunow	-	-	*	*
DIVISION – CYANOPHYTA				
CLASS – CYANOPHYCEAE				
ORDER I – CHROOCOCCALES				
Chroococcus turgidus (Kutz.) Lemm	*	*	-	-
Merismopedia gluca (Ehr.) Nageli	*	-	-	-
Microcystis aureginosa Kutzing		*	*	*
Gleocapsa sp.	*	*	*	*
ORDER II – HORMOGONALES				
Lynbgya limnetica Lemm	-	***	*	*
Lynbgya martensiana Meneghini	-	-	**	*

Oscillatoria sp I.	*	*	-	-
Oscillatoria sp II.	-	-	*	*
DIVISION – EUGLENOPOHYTA				
CLASS – EUGLENOPHYCEAE				
ORDER – EUGLENALES				
Euglena sp.	*	-	-	-
Species diversity (S)	12	13	22	21

Where * represents 1 – 10 cells / colony / Individuals; ** represents 11 – 100 cells / colony / Individuals and *** represents 101 – 4500 cells / colony / Individuals.

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The ecology and natural food components of *Pachymelania aurita* MÜLLER (Gastropoda: Melaniidae) in a Coastal lagoon

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Abstract: The **e**cology and natural food of *Pachymelania aurita* MÚLLER in a coastal Nigerian lagoon are reported. Ecological parameters were investigated by monthly sample collection from September, 2004 to August, 2006. Natural food components were identified by microscopic examination of the feacal matter. The abundance of the *P. aurita* was related to salinity, total organic content (TOC) and grain size of sediment at the study stations. Greater densities of *P. aurita* occurred at stations with relatively higher salinity (>1.77‰), low TOC (<10%), high sand (>60%) and low mud (<30%) contents. A range of 0.01 – 19.72‰ for water salinity was observed during this study, while sediment sand and mud recorded ranges of 65.8-92.8% and 7.8-29.4% respectively. The TOC of sediment ranged between 2.05 and 98.5%. Sediments at the study stations were predominantly sand intermixed with varied proportions of mud and varied rapidly within relatively short distances along the study stretch. A total of 6,869 individuals were recorded during the study, with wet and dry season contributions of 3,693 and 3,176 individuals respectively. Population of *P. aurita* was highest (490 individuals) in the month of January, 2005, while the lowest (230 individuals) was recorded in October, 2005. Total biomass was lowest (55.64g) in March, 2005, while the highest (166.90g) was observed in July, 2005. The natural food of *P. aurita* consists of blue-green algae (*Anabaena, Aphanocapsa*), diatoms (*Navicula, Synedra, Cyclotella, Nitzchia)* bacteria, higher plant materials, organic debris and sand grains. Report and Opinion. 2009; 1(5):41-48]. (ISSN: 1553-9873)

Key words: ecology, natural food, *Pachymelania aurita*, coastal lagoon.

1. Introduction

The genus *Pachymelania* is one of the commonest and most dominant gastropod molluscs in the south-western lagoon systems of Nigeria (Oyeneka, 1975; Uwadiae, 2009; Uwadiae et al., 2009). It is endemic to West Africa (Oyenekan, 1975), and is harvested by natives of coastal towns and villages in Nigeria as a staple source of protein.

Although *Pachymelania* spp adapts to freshwater they prefer brackish water of higher salinity and often extremely abundant in mangrove swamps and on mud-flats within the reach of the tide in the lagoons and river estuaries (Egonmwan, 2007). Of the four species, only *P. bryoensis* inhabits fresh water, others including *P. aurita* and *P. fusca* are characteristics of brackish tidal water and mangrove swamps along the West African Coast (Oyenekan, 1975; Egonmwan, 2007).

The shell characteristics, classification and geographical distribution of the genus have been reported (as cited in Egonmwam, 2007).The ecology of the genus in relation to changes in temperature, salinity and survival out of water under experimental conditions has been documented (Oyenekan, 1975).The genital ducts of three species (*P. aurita, P. fusca, and P. bryoensis*) have been described (Oyenekan, 1984). The production and population dynamics of *P. aurita* in the brackish water Lagos lagoon have been studied by Ajao and Fagade (1990).

Most of the literatures on *P. aurita* in Nigeria is on the high brackish water populations. There is apparently no information on the ecology of fresh water and low brackish water populations. Furthermore, information on the food of *P. aurita* like many benthic invertebrate species is limited, this has left huge gap in the foundational knowledge required if we are to think of domesticating the gastropod to save their populations from complete decimation in the face of the serious ecological threats to their natural habitats.

This paper aims at highlighting the factors affecting the abundance and distribution of *P. aurita* in a low brackish water environment. The paper also reports the natural food of *P. aurita* in the study area.

2.0. Materials and Methods

2.1. Description of Study Area

Epe lagoon (fig. 1) is located in Lagos state. It lies between latitudes $3^0 50' - 4^0 10'$N and longitudes $5^0 30' - 5^0 40'$E. It has a surface area of about 243km^2. The lagoon which has an average depth of about 2.45m is fed by the waters of adjoining rivers and creeks. It is connected to the ocean through the Lagos harbour and tidal influence is relatively weak. An elaborate description of the study area is provided in Uwadiae (2009) and Uwadiae et al. (2009).

2.2. Field Investigation

In order to address the issues regarding the ecology of *P. aurita*, it was important to know the

substratum conditions which determine the occurrence and habitat selection of the organism. Sediment samples were collected using a Van Veen grab ($0.1m^2$) from an anchored boat with an out-board engine. The sediment samples collected at each station were placed in labeled polyethylene bags for grain size and total organic content analysis in the laboratory. The samples were stored in the refrigerator prior to analysis. Three grab hauls for *P. aurita* specimens were also taken from each station, the collected material from two of the hauls were washed through a 0.5mm mesh sieve. The residue in the sieve was fixed in 10% formalin solution and kept in labeled plastic containers for onward transportation to the laboratory. The third haul was emptied into a wide open plastic bowl and specimens of *P. aurita* picked into plastic containers with water from the habitat and transferred to the laboratory. Water samples for the salinity analysis were collected with prewashed plastic bottles.

2.3. Laboratory Investigation

Sediment grain size analysis was performed using the direct method for separating sediment into grain size fractions. Air dried samples were passed through a graded series of standard sieves. Griffin SIH – 310-V sieving outfit was used. The fractions of sand and mud obtained were recorded in percentages. The TOC of the sediment was estimated by loss of weight on ignition in muffle furnace at 555^0C as employed by Uwadiae et al. (2009).

Fixed samples were washed with tap water to remove the fixative and any remaining sediment to facilitate easy removal of specimens of *P. aurita*. The number of individuals for each station were counted and recorded. The changes in population densities of the gastropod within the 24 months period were examined. Salinity was determined according to the methos described by APHA (1985).

2.3.1. Determination of Biomass

The biomass was determined by wet method. This involved direct weighing of all the specimens of *P. aurita* in each sample. They were allowed to dry for one minute after puncturing the shells with a fine needle and the mantle cavity water sucked up with filter paper. The organisms were then weighed using a weighing balance and values approximated to the nearest weight in gramme (g).

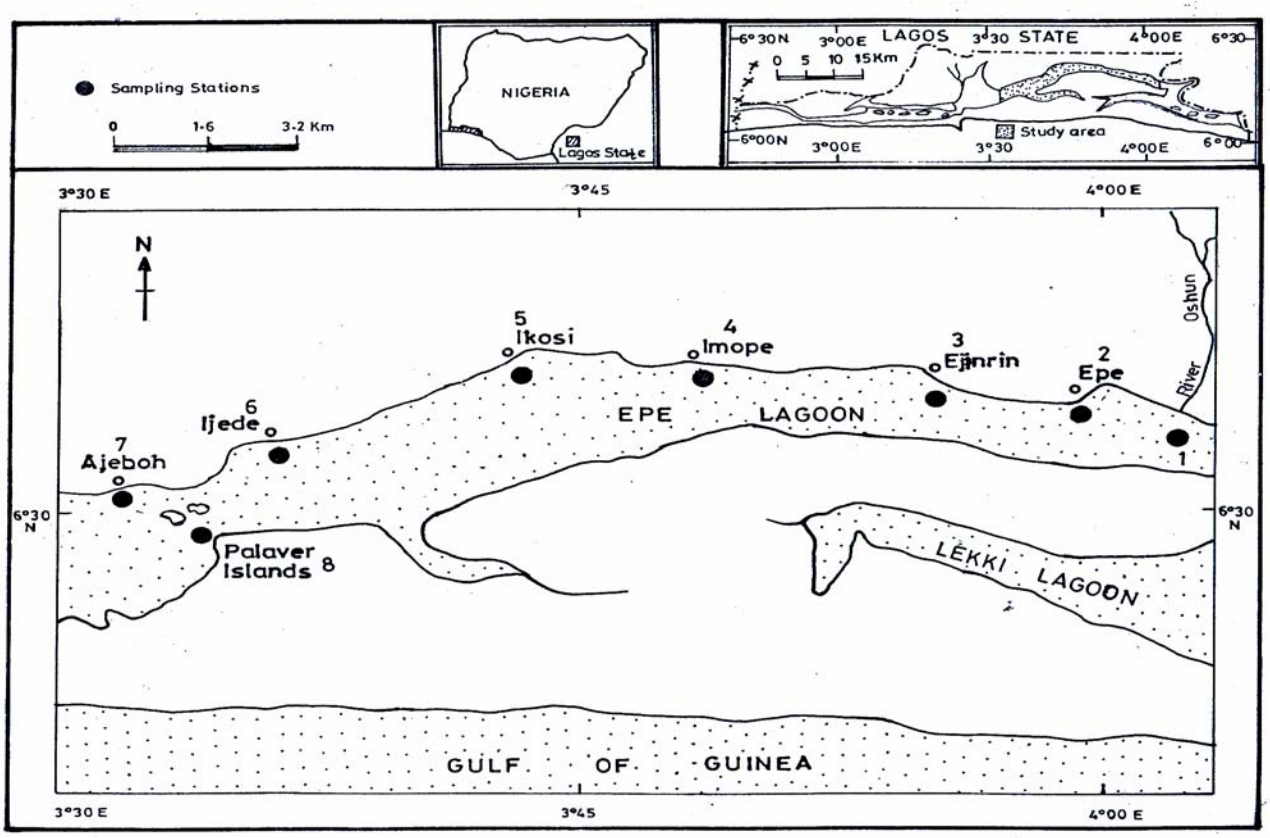


Figure 1. Map showing the study stations

2.3.2. Food Components

The composition of the natural food of *P. aurita* was determined by faecal content analysis as described by Thomas et al. (1985) using the frequency of occurrence and numerical abundance methods (Thomas et al., 1985; Ugwumba, 1990; Ugwumba and Adebisi, 1992). Specimens of *P. aurita* were separated into juveniles (1-10mm) and adults (>17mm). Sixty juveniles and adults were selected on the bases of good health and placed in two different tanks containing sediment and water from the habitat for 72 hours. These specimens were then transferred into Petri dishes (5 specimens of in each Petri dish) with water from the habitat and allowed to stay for 72 hours. The faecal matter of the adults and juveniles were fixed with 30% formalin solution. All the formalin fixed feacal materials were examined under the microscope and the food items identified.

3.0. Results

3.1. Physico-Chemical Characteristics of Sediment.

The summary of values of physicochemical parameters investigated during the study period is presented in table 1. The study area was predominantly sand intermixed with varying proportions of mud. The percentage of sand ranged from 65.8 to 92.8%, and mud fraction ranged between 7.8 and 29.4%. All the stations studied showed fluctuating levels of total organic content. The highest value (98.5%) was observed at station 3 in September, 2004, while the lowest value (2.05%) was recorded at station 5 in December, 2004. Salinity values for the study area ranged between 0.00 – 19.72ppt.

3.2. Distribution, Population and Biomass.

During this study it was observed that *P. aurita* occurred in larger numbers in stations closer to the brackishwater Lagos lagoon. Five of the stations (4 to 8) contained the gastropod in all the samples collected during the sampling months. The gastropod was recorded ten times in station 2, six times in station 3, and three times in station 1. Low numbers (3, 53 and 7) of individuals were recorded in stations 1, 2, and 3 respectively, while stations 4 to 8 recorded higher numbers (723, 1,709, 2,135, 1,422 and 1,491 respectively) of individuals.

Variation in the abundance of *P. aurita in* some of the study stations is presented in Figure 2. The seasonal variation in the population and biomass of *P. aurita* are shown in Figures 3 and 4. The highest population (490) of *P. aurita* in the study area occurred in the month of January, 2005, while the lowest (230) was recorded in October, 2005. In May, 2005, 443 individuals of *P. aurita* were encountered. A reduction in the number of individuals was observed in May, 2006 when 437 individuals were enumerated. Four hundred and twenty five (425) individuals occurred in the samples collected for the month of July, 2006. The results recorded in this study indicate increase in population during the transitional months from rainy to dry season.

Monthly densities of individuals ranged between 144 observed in the month March, 2006 and 544 which occurred in the month of August, 2006. The monthly variation in the number of individuals showed a comparatively higher number of individuals in the transition months from rainy to dry season. The highest density (190/0.1m^{-2}) per sampling effort was recorded in November, 2005 at station 6.

Pachymelania aurita was most abundant in sediments with percentage TOC content between 3.63 and 4.14%, and sand between 78.5 and 81 %. The density was low in sediments with high percentage TOC, such as that in station 3 (Figure 5). Although no particular trend was shown in the biomass values recorded, higher biomass (like the population) values occurred during the transitional months from rainy to dry season. Total biomass was lowest (55.64g) in March, 2005, while the highest (166.90g) was observed in July, 2005.

Table 1. Summary of values of physico-chemical parameters of the study stations.

Parameter	Study station															
	1		2		3		4		5		6		7		8	
	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
Salinity(ppt)	0.35	0.00	0.24	0.00	0.28	0.00	1.77	0.01	3.62	0.01	8.37	0.01	19.30	0.06	19.72	0.04
Sand (%)	92.2	79	87.4	61.4	89.4	54.4	85.4	65.4	93.6	71.4	92.2	73.6	89	65.8	92.4	73.5
Mud (%)	21	7.8	28.6	11.4	44.6	9.4	27.6	14.6	28.6	6.5	26	7.8	29	11	26.5	7.6
TOC (%)	8.61	2.11	8.22	2.10	10.45	3.51	7.50	1.01	7.30	1.02	7.50	1.01	6.00	1.01	6.30	1.01

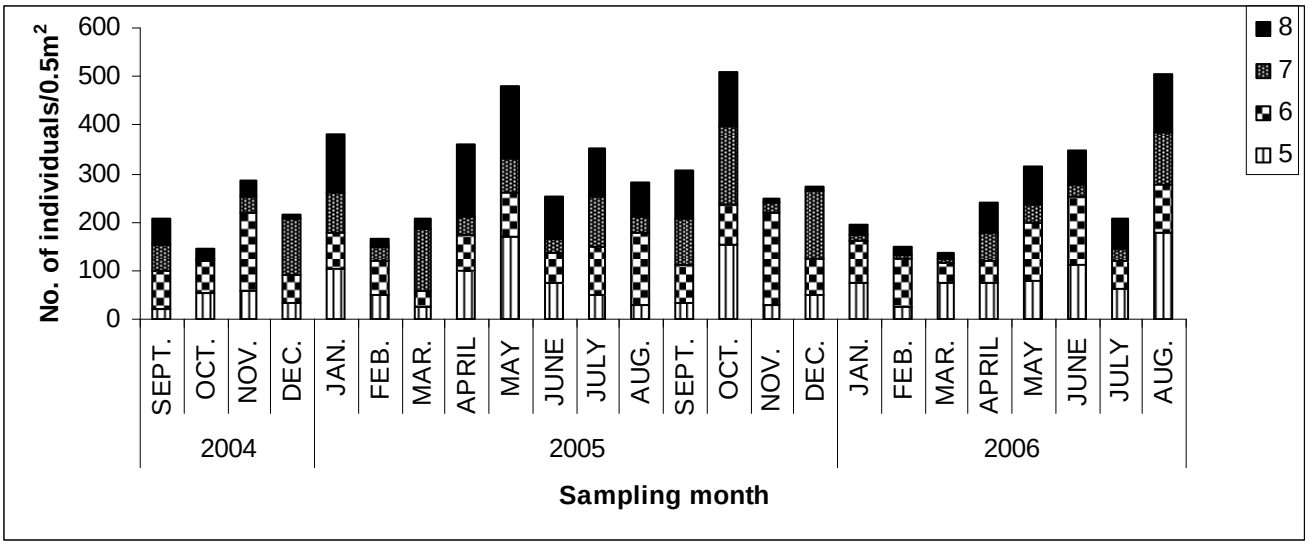


Figure 2: Variation in abundance of *P. aurita* at some of the study stations during the sampling months.

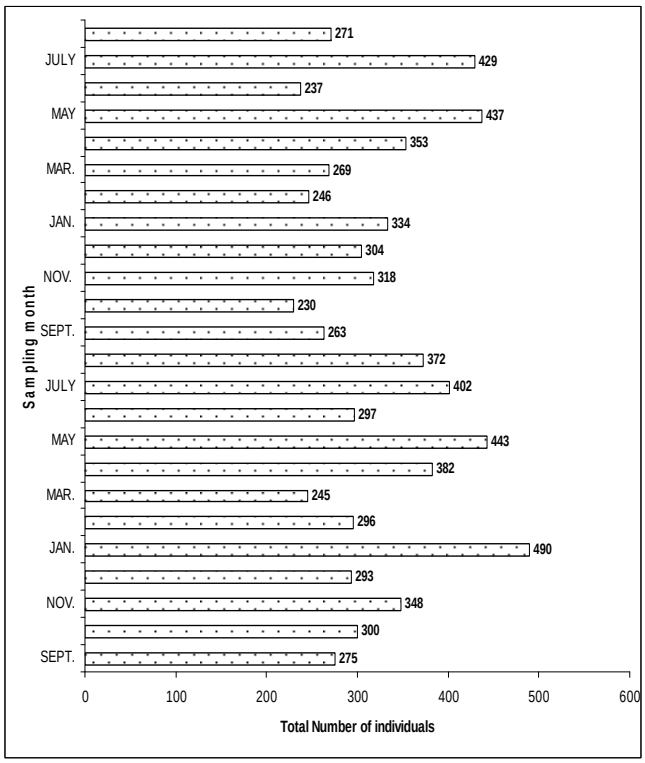


Figure 3. Seasonal variation in the population of *P. aurita* in Epe lagoon (Sept. 2004 - Aug. 2006).

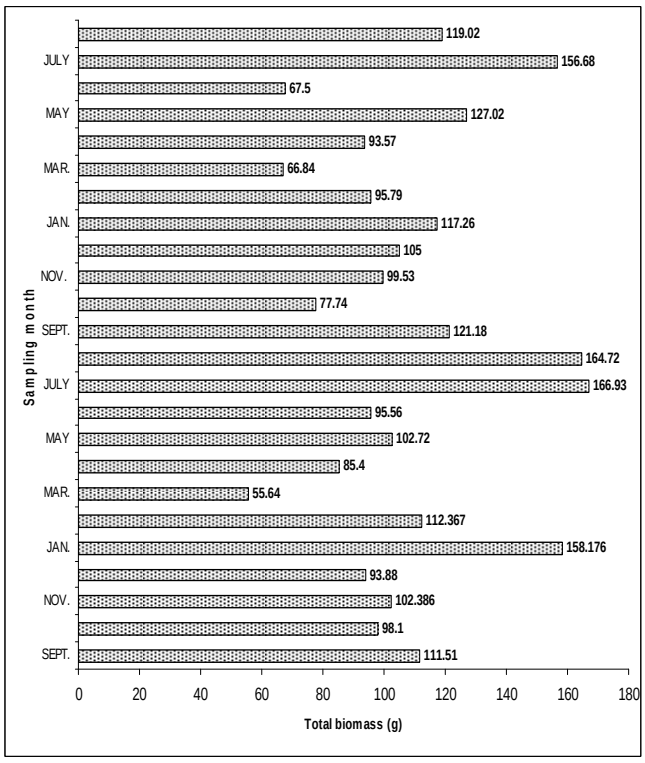


Figure 4: Seasonal variation in the biomass of *P. aurita* in Epe lagoon (Sept. 2004 - Aug. 2006)

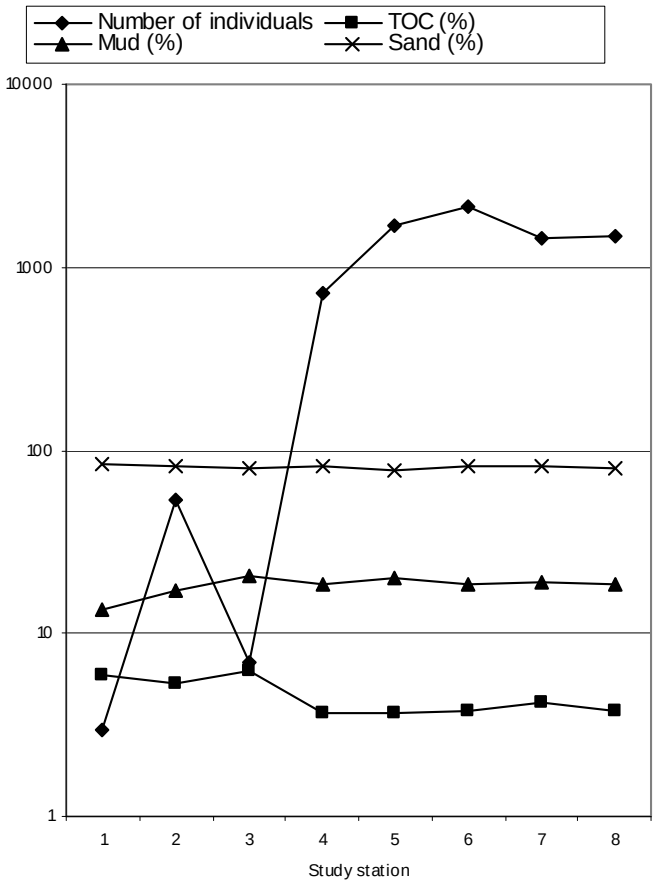
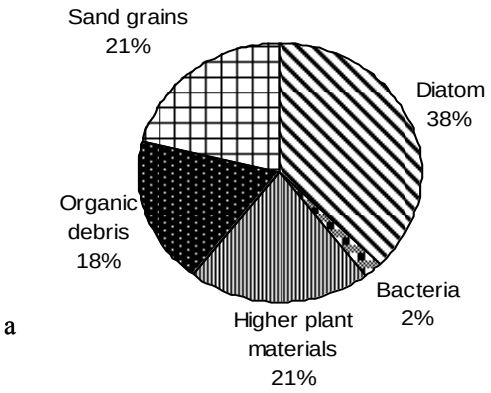


Figure 5: Variations in percentage TOC, sand, mud and number of individuals at the study stations.

3.4. Food Components

Figure.6 shows the percentage composition of the food items in the faecal matter of *P. aurita*. Items encountered in the faecal matter of the specimens examined included microalgae, bacteria, vascular plant materials, organic debris and sand grain. Items found in the faecal matter of adults but not in the faecal matter of the juveniles included a diatom *Cocconeis*. In terms of the frequency of occurrence, higher plant materials, organic debris and sand grains ranked highest (100%), they occurred in all the specimens examined. In the adults of *P. aurita*, the diatoms, *Navicula* and *Synedra* ranked second in percentage occurrence with their presence in 75% of the total adults. In the juveniles *Synedra* and *Cyclotella* ranked second in percentage occurrence, they were represented in 75% of the total juveniles.

In terms of numerical abundance or percentage number, higher plant materials and sand grains constituted the highest number (21.43%) in the faecal content of the adult of *P. aurita* from the habitat. Organic debris constituted 18.57%, *Navicula* 11.42%, *Synedra* 10%, *Cyclotella* 8.57%, *Niztchia* and *Cocconeis* recorded 5.71% and 4.2% respectively. In the juveniles of *P. aurita* from the habitat, sand grains constituted the highest percentage number (25%). Organic debris and higher plant material constituted 22.22% and 19.44% respectively. *Navicula* recoreded 13.88%, *Synedra* 11%, *Cyclotella* 8.33% and *Niztchia* 5.55%.



a

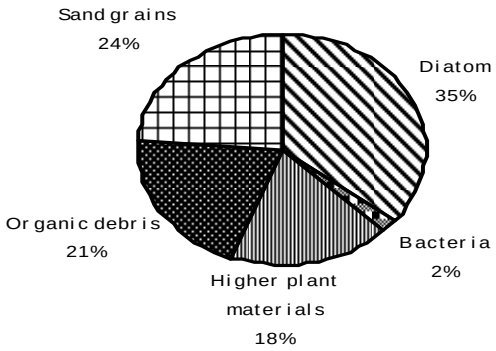


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Figure 6: Percentage composition of the faecal matter of specimens of (a) adult, and (b) juveniles of *P. aurita*.

4.0 Discussion

The narrow variability of sand and mud observed in the study area corroborates the results recorded by Oyenekan (1988) on the sediment characteristics in the Lagos lagoon. Sediment in the study stretch was sandy, except for station 3 where the proportion of mud was more than sand particles in the months of September and December, 2004 and January, 2005. The TOC recorded in all the stations ranged between 2.05 and 8.5%. This is in close semblance with the result (0.50 – 11.95%) reported for the Lagos lagoon by Brown (2000), but differs from that (53.21 - 90.18%) reported by Ajao and Fagade (1991) for the same lagoon. The lowest value of TOC observed occurred at station 5 during the dry season and may be attributed to low sediment transport and reduced allochthonous input. The highest value recorded occurred in station 3 in October, 2004 and could be due to deposition of organic debris from the farm land by this station (Uwadiae et al., 2009).

The distribution of *P. aurita* in the study area is a reflection of its euryhaline nature. It occurred in all study stations. This observation is similar to that of Ajao and Fagade (1990) which opined that the organism was recorded in most part of the Lagos lagoon all the year round and was relatively tolerant of physical and chemical variations in the environment. Like most aquatic molluscs (Eckman, 1983) it however prefers sediments with little silt. The low density and complete absence of the species in some grab samples in some study stations (1, 2 and 3) is associated in part with the possible effects of anthropogenic activities and majorly to salinity of the stations (Uwadiae, 2009). According to Egonmwan (2007), although *Pachymelania* spp adapts to freshwater they prefer brackish water of higher salinity. Of the four species, only *P. bryoensis* inhabits fresh water, others are characteristics of brackish tidal water and mangrove swamps along the West African coast (Oyenekan, 1975; Egonmwan, 2007). This explains the preponderance of the species in stations (4 to 8) where higher salinity values were observed. The euryhaline nature of the organism is a possible premise while there were higher numbers of individuals observed during transiting months from rainy to dry season.

Station 3 where the highest mud content was observed in the substratum, recorded the least number (7) of *P. aurita*. Although muddy particles hold the largest amounts of total TOC which represent a food source for deposit feeding organisms (Brown, 2000), it limited the abundance of the filter feeder. Induced sedimentation resulting from high organic matter can smother benthic molluscs both at their adult and planktonic stages. Increased turbidities may increase the formation of pseudofeces and decrease the amount of water that is pumped (Hart and Fuller, 1979).

The highest biomass value (666.285g) occurred in station 5. This may be attributed to the seemingly favourable environmental conditions prevalent in this site. These include a relatively high sand content with proportionate admixture of mud and organic matter. The results of biomass recorded in this study compares favourably with those of Ajao and Fagade (1990), who reported lowest and highest values of annual biomass of *P. aurita* as 47.513mg and 7082.7646mg respectively.

The detrimental consequences of anthropogenic activities particularly with respect to the introduction of organic matter, waste dump and sand mining also limited the abundance of the species. This observation was supported by the occurrence of species associated with organic pollution at some of the study stations. These included *Nereis* spp and *Chironomous* sp which have been reported in similar disturbed environments (Chukwu and Nwankwo, 2004) and referred to as opportunistic species.

z Information on the food of *P. aurita* is limited. According to Calow (1970), little detailed information concerning the natural diets of aquatic gastropods is available. This work therefore is among the first major report on the food of benthic gastropod in a tropical lagoon. Analysis of the faecal content of *P. aurita* shows that its food items include miroalgae, bacteria, vascular plant materials, organic debris and sand grains. This array of food items is similar to those recorded as stomach contents of some aquatic gastropods (Cummins, 1979; Thomas et al., 1985; Akintunde, 1988; Ugwumba, 1990; Egonmwan, 1991). The preponderance of sand grains in the faecal matter of all the specimens examined corroborates the reports of Thomas et al. (1985) and Dillon (2000). Organic debris ranked second in terms of percentage abundance and occurred in all the animals examined. Benthic microalgae are embedded in a complex sediment structure, so grazers move through the interstitial system or upon the surface and capture mobile flagellates and diatoms, or also browse the epigrowth on sand grains (Dillon, 2000). Also, sand grains are commonly found in the stomach of most freshwater gastropods, constituting an especially large fraction of the gut contents in many lymnaeids (Dillon, 2000). It has also been reported that sand grain is actively ingested and used in the tituration of food (Dillon, 2000; Graham, 1955).

The occurrence of diatoms and cyanobacteria as the only microalgae encountered in this study may be linked to their preponderance in aquatic sediment (Nwankwo and Akinsoji, 1989). Their survival in may linked to their habit and general biological characteristics. A number of species of cyanobacteria and diatoms are adapted to life in the aquatic sediment (Dakshini and Soni, 1982). Nwankwo and Akinsoji (1989) reported the greatest count of diatoms notably *Navicula* and *Nitzichia* in their study of the benthic algal community of a saw dust deposition site. They also stated that diatoms are the dominant plants in mud and sand. The overwhelming presence of the diatoms among other microalgae in the faecal matter of *P. aurita* may be attributed to their relatively indigestibility caused by the impregnation of their cell **w**all with silica and the overlapping of the two halves of the cell wall making it difficult for the simple digestive system of the animal to cope with. However, it has been reported that some benthic species are able

to puncture and suck out the content of diatom cells (Dillon, 2000).

No living macrophyte or other living plant material was identified as a component of the feacal content of *P. aurita*, aquatic snails tend not to attack living macrophytes under natural conditions. Thomas et al (1985) reported that snails under natural conditions consume much higher quantities of dying or decomposing aquatic plants than living materials. This claim is corroborated by the findings of Scheerboom and Van Elk (1978; Thomas et al., 1983) which showed that detritus (mainly of macrophyte origin) was the major dietary item for *L. stagnalis* as it formed 48.3% of the mean percentage composition of crop contents. Dead plant materials are also the major item in the diet of most species of terrestrial molluscs as well as freshwater prosobranchs such as *Viviparus contectus, Bithynia tentaculata* and *Melanopsis* spp (Dillon, 2000). In view of the predilection of most prosobranchs for dead or senescing plant material the percentage occurrence of organic debris recorded in this study for all the categories of specimens is true. Much of the organic debris recorded were in their early stages of decay which the animals might be able to take into their system during feeding activities.

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Standardization of cultural conditions for maximum vanillin production through ferulic acid degradation

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Abstract: Work has been carried out to study the standardization of different cultural conditions during the biotransformation of ferulic acid into vanillin using a Streptomyces isolate S10. Three parameters such as substrate concentration, temperature and supplementation of other carbon source (glucose) were taken into consideration. Ferulate concentrations of 5 mM, temperature of 28° C were standardized. Addition of glucose showed 5- fold increase in vanillin production. [Report and Opinion. 2009; 1(5):49-51]. (ISSN: 1553-9873).

Key words: ferulic acid, biotransformation, vanillic acid, vanillin, *p*-coumaric acid

1. Introduction

Hydroxycinnamic acids such as ferulic acid and *p*-coumaric acid occur widely in the cell walls of graminaceous plants (Grabber *et al*. 1995; Harris and Hartley, 1980). Ferulic acid is a very important component for the structure and the biology of cell-wall as it can cross link polysaccharide chains through dimerisation reaction (Ishii, 1997). Microbes transform hydroxycinnamic acids to their corresponding hydroxybenzoates. These benzoates are important components of natural flavours and fragrances. A number of industrial and food applications were reported for ferulic acid, especially based on its microbial degradation to vanillin. Vanillin is the world's most highly prized natural flavour. It is one of the most important aromatic flavour compounds used in foods, beverages, perfumes and pharmaceuticals (Clark, 1990). Thus, considering the increasing interest in 'natural' products, the production of flavours via biotechnological processes offers a viable alternative to natural and chemical sources (Walton *et al*. 2003). This work reports the capability of *Streptomyces* isolate S10 to degrade ferulic acid. In this process of biotransformation, vanillin was the major degradation product. Various parameters such as substrate concentration, temperature and supplementation of other carbon source (glucose) were analyzed for maximum vanillin production.

Materials and Methods

Microorganism

Streptomyces **S10** was isolated from soil on the basis of its ability to grow in ferulic acid containing medium. Pure cultures of these strains were maintained on Arginine Glycerol Salt (AGS) slants **(**El-nakeeb and Lechevalier**,** 1963).

Medium and Culture conditions

After growth on AGS broth for 7 days, 1 ml cell suspension was transferred into the 100 ml flask each containing 25 ml of minimal medium (Muheim and Lerch, 1999) containing ferulic acid as a sole carbon source. The pH of the media was adjusted to 7.0. The cultures were incubated at 28^0C and analyses were carried out on day-to-day basis upto 8 days of incubation to detect the degradation product of ferulic acid. Each experiment was carried out in triplicate. The standard deviations of the analyses were less than 5%.

Extraction and detection of metabolites from the culture media

For the extraction of ferulic acid and its degradation product from the culture media, culture supernatants were prepared by centrifugation. These were acidified (pH 1-2) and extracted with equal volume of ethyl acetate. The ethyl acetate was evaporated in vacuum and residue was re-dissolved in 50% methanol. This processed culture filtrate was subjected to thin layer chromatography (TLC) and HPLC. (Ghosh *et al*. 2005; Sachan *et al*. 2004). Further confirmation was carried out using the Electron Spray Ionization Mass Spectrometric (ESI-MS).

Standardization cultural conditions

a. Concentration:

Effect of various concentrations of ferulic acid on vanillin formation was examined by flask experiments. Microorganisms were grown aerobically in minimal media containing various concentrations (1.0, 2.5, 5.0, 7.5, 10.0 mM) of ferulic acid as a sole carbon source. After 8 days of incubation, the ferulic acid utilization was carried out.

b. Temperature

Cultures were incubated at various temperatures (28 ^{o}C, 37 ^{o}C). Day basis analysis was carried out by sampling the cultures for 8 days.

c. Supplementation of other carbon source (Glucose)

In order to make high density culture of *Streptomyces* isolate S10, microorganism was allowed to grow in minimal media supplemented with glucose (0.1% w/v) as an sole carbon source. After completely consumption glucose by the microorganism, ferulic acid (5.0 mM) was added into the minimal medium.

Results and Discussion

In this case study, 5mM concentration of ferulic acid was found to be optimum for maximum vanillin production (**Fig. 1).** It was observed that a maximum amount of vanillin (10.334 mg/l) was obtained on 7th day of incubation at 28 ^{o}C **(Fig 2, Table. 1)**. In this case, microorganism consumed ferulic acid very quickly with maximum accumulation of vanillin (51.865 mg/l) after 12 h **(Fig 3, Table. 2)**. There was an increase in product (vanillin) accumulation with increase in concentration of ferulic acid up to 5mM concentration. With further increase in concentration (7.5 mM and 10 mM), there was a decrease in the product formation. The optimum temperature required for maximum vanillin production was 28 °C. A maximum amount of vanillin (10.33 mg/l) accumulated on day 7 of incubation at 28 °C in comparison to the vanillin (4.2 mg/l) production at 37 °C. Supplementation of other carbon source (glucose) at 0.1% (w/v) concentration along with ferulic acid was tested as another cultural condition for maximum amount of vanillin production. It was reported earlier that the use of additional carbon source helped in the formation of high density cultures (Oddou et al. 1999) which helps in the formation of product in a shorter period of incubation period. Microorganism consumed ferulic acid very quickly with 5 fold accumulation of vanillin (51.865 mg/l) after 12 h. In this bioconversion process in presence of glucose, vanillic acid was also detected along with vanillin. It was assumed that the amount of vanillin that accumulated in the culture medium was probably toxic for the microorganism, hence was further converted to vanillic acid in this case.

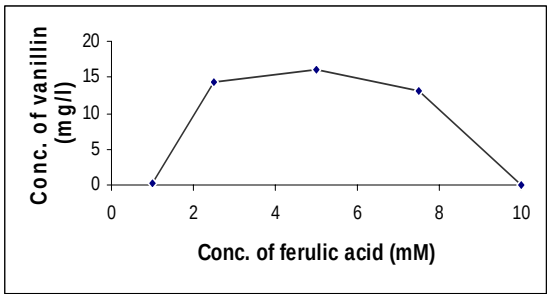

Figure 1.Time course accumulations of vanillin in the culture media of *Streptomyces* isolate **S10** at various concentrations of ferulic acid (mM).

Table 1: Time course detection of vanillin in the culture media of *Streptomyces* isolate **S10** at two different incubation temperatures.

Day	Conc. of vanillin at 28°C	Conc. of vanillin at 37°C
1st	0.00	0.00
2nd	0.483	0.109
3rd	2.481	0.264
4th	3.586	1.495
5th	3.941	3.907
6th	5.902	4.199
7th	10.334	3.814
8th	8.631	3.138

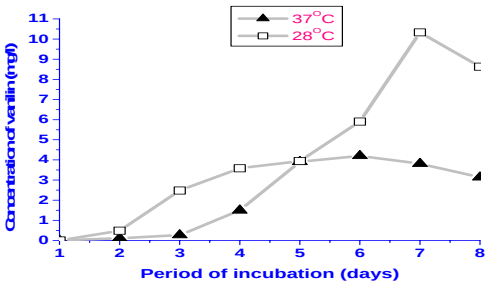

Figure 2. Time course detection of vanillin in the culture media of *Streptomyces* isolate **S10** at two different incubation temperatures.

Table 2. Time course degradation of ferulic acid (FA) and detection of vanillin (Van) and vanillic acid (VA) in the culture media of *Streptomyces* isolate **S10**.

Hours	FA (mg/l)	Van (mg/L)	VA (mg/l)
12 h	188.804	51.865	29.574
24 h	172.402	10.558	17.327
48 h	2.320	6.376	6.4
72 h	0.088	4.610	5.6

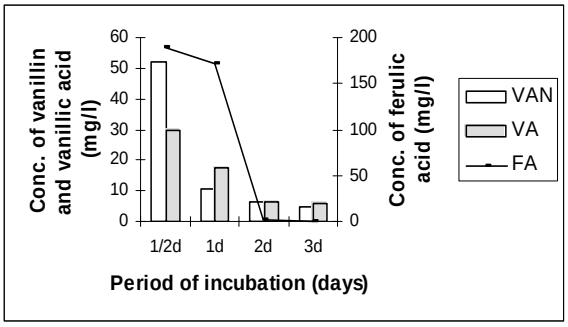

Figure 3. Time course degradation of ferulic acid and detection of vanillin and vanillic acid in the culture media of *Streptomyces* isolate **S10**.

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Role of Fulvic Acid on Reduction of Cadmium Toxicity on Nile Tilapia *(Oreochromis niloticus)*

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Abstract: Cadmium is one of the most toxic heavy metal, enters the environment from natural sources and as a result of mans activity. Tilapias have the capability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues. The reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method. The effect of fulvic acid (FA)on cadmium (Cd) toxicity and the impact on fish immunological, hematological changes in Nile tilapia (*Oreochromius niloticus*) were studied. The fish (100±10g)were exposed to 10 ppm Cd alone or with 0.1, 0.2 and 0.3 ppm for 15 and 45 days. Cd exposure reduced significantly ($P<0.04$) such as erythrocyte count (RBCs), haemoglobin content (Hb), haematocrit value (Hct), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration. These parameters were improved when FA was applied with Cd. The values of RBCs, Hb, Hct, MCH and MCHC were increased significantly to be as in the control fish group. Addition of FA to Cd contaminated medium considerably reduced metal absorption and accumulation in fish tissues, while it was increased metals in water and feces. The present study, is recommended that an optimum dosage of 0.3 g fulvic acid /l can effectively chelate Cd from contaminated fish and water. [Report and Opinion. 2009; 1(5):52-57]. (ISSN: 1553-9873).

Key words: Nile tilapia, Cadmium, immunological, fulvic acid, hematology, liver, gills ,musculature.

1-Introduction

Nile tilapias are considered the most popular widely distributed cheapest and intensively cultured fishes in Egypt. Cadmium is one of the most toxic heavy metal, enters the environment from natural sources and as a result of mans activity e.g. There cycling of scrap metal, electroplating industries manufacturing vinyl plastics, electrical contacts, metallic and plastic pipes. Tilapias have the capability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues. The reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method. The most widely used technique for the removal of toxic elements involves the process of neutralization and metal hydroxide precipitation, Hiemesh and Mahadevaswamy (1994). Chemicals can effectively remove certain toxic elements from industrial wastes or polluted media, but is usually costly. However, there are some cheap natural products which are also free from undesirable side effects. In recent years, the remobilization of metals by synthetic anthropogenic chelating agents has received much attention. The literature reported number of chelators that have been used for chelate-induced hyper accumulation, Khangarot and Tripathi (1991). Natural compound like FA is known to be effective chelating agents of heavy metals, Karuppasamy et al,2005. FA is the most commonly used cheater because of its small molecular weight and strong chelating ability for different heavy metals ,Litchfield and Wileoxon (1949) and Donor and William (1993) . Metal bioaccumulation can occur via complication, coordination, chelation, ion exchange and other processes of greater or lesser specificity. Bioaccumulation processes are sometimes due to active (metabolism dependent) metal accumulation by living cells, Aiken et al,1985. In spit of the amount of data published on the effect of water borne exposure of cadmium and FA singly, information on the effects of Cd / FA mixture on aquatic organisms are limited and not uniform. Therefore, FA appears to be promising tool to control cadmium pollution in aquaculture Murry and Linder (1983). The present study, short and long– term bioassays were designed to evaluate the influence of FA on the retention of cadmium in water. It was carried out to investigate the effect of FA on reduction of toxicity of cadmium for enhance the change of blood parameter and enzymes and to assess its impact on some physiological parameters of Nile tilapia *(Oreochromis. Niloticus)*.

2-Material and methods

2-1-Collecting sample Tilapia

A healthy 75 fish of Nile tilapia Oreochromis niloticus weighing (100±10g)/ fish were collected from the ponds of Kafr Eel Sheikh governorate fish farms, Egypt. Fish were acclimated in cement fish ponds for 2 weeks. Acclimated fish were exposed to different concentration of cadmium and mortality were observed for 96- h. A static renewable bioassay method, Duncan (1955) was adopted for the determination of 96-h median lethal probity analysis, Santschi (1988) was followed the calculation of 96 hr LC50. A control group was maintained in metal – free tap water. The 96 hr LC50 of cadmium for Oreochromius niloticus was 40 ppm. A stock solution of cadmium was prepared by dissolving 10.686 g 0f annular grad cadmium sulphate (CdSO4– 8/3H2O) in 1/L of distilled water and the diluted with water to obtain the desired concentration (10 ppm) for this experiment. The fish were distributed randomly in 5 cement pond at a rate of 15 fish / aquarium that containing aerated tap water. These aquaria were divided into five groups with three replicates each per group. Fish were fed frequently a diet containing 25% crude protein (CP) at a rate of 3% of live body weight twice daily for 15 and 45 days. Siphoning three quarters aquariums was done every day for waste removal and replacing it by an equal volume of water containing the same concentration of Cd and FA . Dead fish were removed and recorded daily.

2-2-Sample classification

The first group was free of Cd and FA acid and maintained as a control. The second groups were exposed to 10 ppm of Cd SO4 only. The third, fourth and fifth group were exposed to 10 mg Cd /L and 0.1, 0.2 and 0.3 g FA /L, respectively. Each aquarium was supplied with compressed air via air-stones from air pumps. Well-aerated water supply was provided from a storage fiberglass tank. The temperature was adjusted at 27□1 oC by means of thermostats Table (1).

2-3-Cd residue :

Cadmium sulphate and fulvic acid was obtained from El- Nasr chemical and a Grotech companies (Egypt) respectively, and prepared in aquatic solution to provide the required concentrations of cadmium and fulvic acid. Cadmium was measured in water, liver , gills, muscle and feces according to method of Norvell(1991).

2-4-Statistical Analysis:

The obtained data were subjected to analysis of variance between means were done at the 5% probability level, using Duncan' s new multiple range test by Spraggue(1973).

3-Results

The present study show that addition of FA to Cd contaminated media, reduced significantly the Cd level in the water and helped to eliminate metal from the fish body(liver, gills, musculature and feces) and and in turn improved the biochemical parameters as compared to fish exposed to Cd alone in Table (2).

3-1-Clinical examination:

The clinical examination of most examined fishes showed asphyxia, some aggregated on the surface, accumulated at the water inlet of the pond and air pump of aquaria. Others appeared dull with loss of escape reflex .

3-2-Haematological parameters:

The results of erythrocyte count (RBCs), haemoglobin content (Hb) and haemetocrit value (Hct) obtained from the fish exposed to sublethal dose of Cd (10 mg/l) alone or with different doses of fulvic acid are given in Table (4). Table 3 shows that the RBCs, HB and HCt were reduce in fish exposed to Cd at both periods and they were less than that of the control ($P<0.05$) The RBCs count decreased significantly in fish exposed to Cd at 15 and 45 days. On the other hand, these parameters were return to the normal values and increased significantly in fish exposed to Cd with 0.2 and 0.3 g of FA /l for 15 and 45 days. These values increased significantly in fish exposed to Cd with 0.3 g FA /l. Blood parameter were improved in fish exposed to Cd with different levels of FA . The blood indices calculated from the mean values of blood parameters for the aforementioned treatments are given in Table(2). Data shows that the MCV increased significantly in fish exposed to Cd alone, while the MCH and MCHC

decreased significantly in fish exposed to Cd only when compared with the control. These parameters increased with increasing of exposure time of fish to Cd. Addition of FA to Cd- polluted media maintained the MCV, MCH and MCHC at levels close to those of the control.

Table 1: Field experimental groups and their notation.

S. No.	Groups in field ponds	Nation
1	Control (metal free water)	C
2	Cadmium (10 ppm) alone	Cd
3	Cadmium (10 ppm) +0.1g fulvic acid /l	Cd fulvic acid 1
4	Cadmium (10 ppm) +0.2 fulvic acid /l	Cd fulvic acid 2
5	Cadmium (10 ppm) +0.3g fulvic acid /l	Cd fulvic acid 3

Table 2: Changes in mean cell volume (MCV) , mean celll haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in the blood of Nile tilapia (O.niloticus) exposed to Cd with or without fulvic acid (FA).

Items	MCV		MCH		MCHC	
Period	15 days	45 days	15 days	45 days	15 days	45 days
Control	95.32 ad	100.02 a	34.35 a	43.21 a	34.77 a	43.32 a
	± 1.86	± 2.243	± 0.342	± 1.432	± 1.121	± 0.928
Cd	106.93 b	106.75 b	33.02 b	36.56 b	31.15 b	37.27b
	± 2.23	± 0.874	± 0.177	± 0.846	± 0.909	± 1.85
Cd+0.1 g	93.45a	95.71 a	34.52 a	32.45 b	37.21a	33..77 b
FA/l	± 2.05	± 4.26	± 1.23	± 1.17	± 1.26	± 1.49
Cd+0.2 g	96.24a	98.77 a	33.23 a	36.76 bc	34.06 ac	37.40 b
FA/l	± 2.64	± 0.909	± 1.72	± 1.49	± 1.76	± 1.20
Cd+0.3 g	101.1db	107.95 b	32.85 a	37.02 ac	33.34 cb	34.87 b
FA	± 2.512	± 2.241	± 1.702	± 1.576	± 0.941	± 1.68

Table 3: Changes in cadmium residue in water (mg Cd/L), liver, gills, muscle and feces (mg Cd/g dry weigh) of Nile tilapia (O. niloticus) exposed to to Cd with or without fulvic acid (FA).

Items	Water	Liver		Gills		Muscle		Feces	
Period/days		15	45	15	45	15	45	15	45
Control	0.041	0.048 a	0.055 a	0.038 a	0.039 a	0.023 a	0.076 a	0.003 ab	0.005 ab
	± 0.02	± 0.02	± 0.004	± 0.02	± 0.04	± 0.002	± 0.005	±0.018	± 0.02
Cd	9.31	2.15 b	5.971 b	1.36 b	2.56 b	0.476 b	1.077 b	0.153 b	0.189 b
	± 0832	± 0.253	± 0.85	± 0.085	± 0.276	± 0.06	± 0.15	± 0.018	± 006
Cd+0.1g	7.15	1.292 c	4.16 b	0.65 c	1.07 c	0.343c b	0.665 c	0.940 c	2.067 c
FA /l	± 0.34	± 0.056	± 0.45	± 0.06	± 0.11	± 0.04	± 0.021	± 0.03	± 0.143
Cd+0.2g	3.78	0.95 d	3.791 b	0.394 d	0.85 c	0.33 cb	0.383 d	2.34 d	5.443 d
FA/l	± 0.01	± 0.054	± 0.29	± 0.052	± 0.06	± 0.08	± 0.034	± 0.069	± 0.345
Cd+0.3g	1.73	0.42 e	2.45 c	0.266 d	0.71 c	0.216 c	0.217 d	5.282 e	7.456 e
FA/l	± 0.02	± 0.034	± 0.23	± 0.073	± 0.42	± 0.03	± 0.025	± 0.32	± 0.528

Table 4: Changes in erythrocyte (count x 106/mm3), hemoglobin content (g/100ml) and haematocrit value (%) in the blood of Nile tilapia (O.niloticus) exposed to Cd with and without fulvic acid(FA) .

Items	Erythrocyte count (RBCs)		Hemoglobin (HB)		Haematocrit value (Hct)	
Period	15 days	45 days	15 days	45 days	15 days	45 days
Control	1.58 a	1.714 a	5.48 a	7.315 a	15..31 a	17..32 a
	± 0.073	± 0.051	± 0.353	± 0.133	± 0.308	± 1.665
Cd	1.268 b	1.06 b	4.21 b	4.12 c	13..5 b	12.01 b
	± 0.064	± 0.073	± 0.235	± 0.354	± 0.47	± 0.576
Cd+0.1g	1.572 a	1.57 d	4.54 ab	5.12 b	14.66 a	15.05 a
FA/l	± 0.064	± 0.023	± 0.395	± 0.136	± 1.454	± 0.76
Cd+0.2g	1.56 a	1.786 ac	5.17 ab	6.605 b	15.02 a	17.65 a
EDTA/l	± 0.086	± 0.032	± 0.458	± 0.305	± 1.72	± 0.916
Cd+0.3g	1.956 c	2.01 c	6.464 c	7.68 a	20.0 c	22.02 c
FA/l	± 0.086	± 0.063	± 0.277	± 0.133	± 0.365	± 1.471

DISCUSSION

The clinical picture in naturally infested and polluted Tilapia sp were revealed some aggregated on the water surface, accumulated at the water inlet of the pond and air pump of aquaria. Almost, appeared dull with loss of escape reflex, Eissa (1994) and Eaton and Stinson (1983).The present study reveals

that the fish exposed to Cd alone showed significant reduction in RBCs, Hb and Hct than those exposed to Cd with different level of FA. The reduction of these parameters in Nile tilapia, O niloticus at sublethal levels of cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemsynthesis that affected by pollutants, James and Sampath, K (1999) . Also, the decrease in RBCs count may be attributed to haematopathology or acute haemolytic crisis that results in sever anemia in most vertebrates including fish species exposed to different environmental pollutants, Yamawaki (1986) or may be the decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in sever aneamia, Wintrobe (1978). Mousa(1999)found a significant decreased in total erythrocyte count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, Channa punctatus after exposure to sublethal dose of Cd (29 mg Cd/L).The addition of FA improves the haematological parameters (RBCs, Hb and Hct) which indicating to the capability of FA to chelate Cd from the media. Subsequently, the Cd toxicity was reduced. These results are in agreement with those of Snedecor and Cochran, W. G. (1982) who observed that Oreochromis mossambicus exposed to copper along with fulvic acid showed a significant improvement in blood parameters over those copper alone. The perturbations in these blood indices (increase MCV, decrease of MCH and MCHC) may be attributed to a defense against Cd toxicity through the stimulation of erythropiosis or may be related to the decrease in RBCs, Hb and Hct due to the exaggerated disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sublethal concentration of pollutants, Hung et al, 1997. The present results indicate that fulvic acid is effective in removing Cd from water, and reducing Cd bioaccumulation in fish. Particulate organic matter which can scavenge metal from water and help to reduce metal from fish. These results are in agreement with Shalaby (2001) who study that any agent that can remove Cd from water helps to reduce the bioaccumulation of this metal in fish.The present study showed that the addition of fulvic acid to the Cd media reduced significantly ($P<0.05$) the Cd level in water and metal uptake as compared to fish exposed to Cd alone. The Cd concentration in water was 9.31 mg/L and it decreased significantly ($P< 0.05$). The Cd accumulation in liver, gills and muscle of fish exposed to Cd alone was higher than that of FA. These results suggest that FA could chelate Cd ions producing a stable complex, thus reducing the chance for metal uptake by tissues. Besides , the fulvic acid eliminated more amount of Cd from the body through feces. The formation of Cd- FA complex in water and elimination of more amount of Cd in feces evidently reduced the metal burden in tissues and thereby improved the haematological parameters of fish exposed to Cd. Planas-bohne(10) found low level of cadmium in tissues due to increased excretion of metals through feces and urine when rats were administered Cd intravenously along with FA .From the present study, it is recommended that an optimum dosage of 0.3 g FA /l can effectively chelate Cd from contaminated water . Hence, a scientific method detoxification is essential to improve the health of fish in any stressed environmental conditions.

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Synthesis, Spectroscopic, Thermodynamic and Dyeing Properties of Disperse Dyes Derived from 2-Amino-4-Trifluoromethylbenzothiazole

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ABSTRACT

Monoazo disperse dyes were synthesized using heterocyclic amine by the process of diazotization and coupling. The dyes were synthesized by coupling different components to 2-amino-4-trifluoromethylbenzothiazole. The coupling components used are N,N-dimethylaniline, N,N-diethylaniline, bis(2-cyanoethyl)aniline, 3-acetylamino- N, N-diethylaniline, 5-acetylamino-2-methoxy-N,N-diethylaniline, 2-Naphthol and 1-Naphthol respectively. The different properties of the dyes such as yield, melting point, molecular weight, molecular structure, molar extinction coefficient, degree of exhaustion and wash fastness properties on nylon were analysed. The thermodynamic parameters of the seven dyes on polyster were also investigated. The results showed that the dyes have very good extinction coefficient and excellent wash fastness, thus can be of commercial significance. The standard affinities of the dyes on polyester varied between 10.29 $kJmol^{-1}$ to 20.60 $kJmol^{-1}$. The implication of the standard affinities is that dye with $\Delta \mu^0$ 20.6$kJmol^{-1}$ has highest % exhaustion. [Report and Opinion. 2009; 1(5):58-66]. (ISSN: 1553-9873).

Key Words: Monoazo disperse dye, Diazotization, Exhaustion, Extinction coefficient, Thermodynamic parameters and Standard affinities

1. Introduction

In the early days of dyestuffs, the majority of dyes were prepared from benzene and naphthalene derived intermediates, including heterocylic dyestuffs such as Mauveine and indigo. However, a recent trend has seen the introduction of an increasing number of heterocycles as dye precursors and nowhere has the impact been felt more than in azo dyes[1-2]. Numerous heterocyclic dyes are now marketed to the extent that no manufacturer can profess to produce a full range of disperse dyestuffs without handling colorants based on heteroaromatic diazo or coupling components. These dyes are characterized also by having generally excellent brightness and high extinction coefficients, relative to azo dyes derived from substituted anilines. The bathochromic shifts observed with these compounds have been attributed to the sulphur atom imparting enhanced polarizability to the π-electron system. Molecular orbital calculations indicate that the sulphur atom d-orbital are not relevant, and it is the increased diene character of the heterocyclic ring that is responsible for the shifts[3]

Most hetarylazo dyes of technical interest for application to textiles are derived from diazo components consisting of five-membered rings containing one sulphur heteroatom and to which a diazotisable amino group is directly attached; the ring may also possess one or more nitrogen heteroatom and be fused to another aromatic ring. These diazo components are capable of providing red to blue disperse dyes that meet the rigorous technical and economic requirements demanded of them by both manufacturer and user. Hence, it was thought to synthesize a series of dyes, prepared from benzothiazole as diazo component, and to examine the dyeing performance of these dyes on nylon fabrics. The relationship between % exhaustion (%E) and the thermodynamic parameter values such as partition coefficient (K), heat of dyeing and standard affinities can be established.

2. Experimental

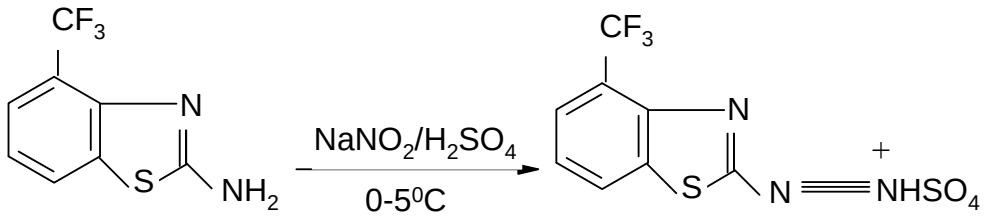
Materials

All the chemicals used for the synthesis of dyes (1)-(7) were of commercial grade. All solvents used were either of analytical grade or redistilled commercial grade.

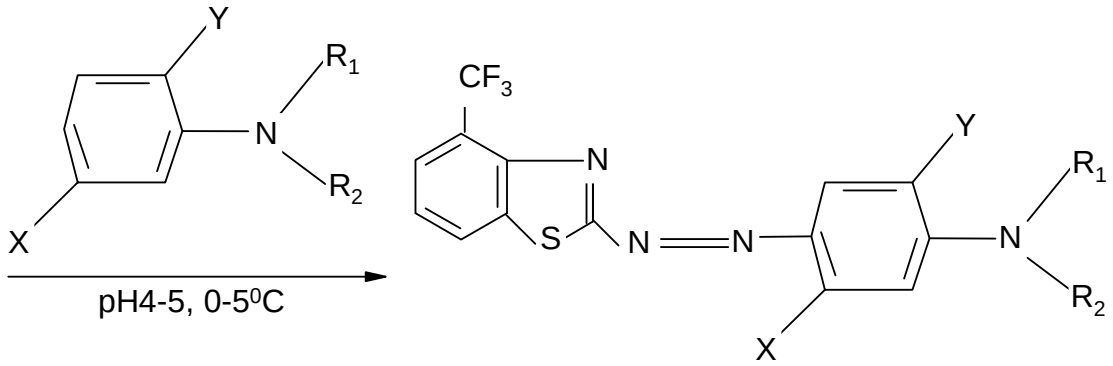
Diazotization of 2-amino-4-trifluoromethyl-benzothiazole (Giles1974).

Diazotization was carried out with nitrosylsulphuric acid. A typical example is as follows. Dry sodium nitrite (1.38g, 0.02 mol) was slowly added over a period of 30 minutes with stirring to concentrated sulphuric acid (1.2ml) which was heated on a water bath to keep the temperature at 65^0C. The solution was then cooled to 5^0C and a mixture (20 ml) of acetic acid-propionic acid (17:3) was added dropwise with stirring, allowing the temperature to rise to 15^0C. The reaction mixture was then cooled to 0-5^0C, and 2-amino-4-trifluoromethylbenzothiazole (0.02 mol) was added

portion wise and stirring was continued at this temperature for 2 hrs. The excess nitrous acid was decomposed with the required amount of urea. The clear diazonium salt solution thus obtained was used immediately in the coupling reaction (see Scehme 1 and 2).



SCHEME 1: DIAXOTIZATION OF BENZOTHIAZOLE



SCHEME 2: COUPLING WITH ARYLAMINE

Coupling

The coupling reaction occurred readily on adding the resulting diazonium salt continuously to a solution of the coupling component in acetic acid. Frequent addition of ice flakes helped to keep the coupling temperature below 5^0C and facilitated the precipitation of the resulting dye. The coupling was usually accompanied by some decomposition; however, by careful addition of the diazonium salt solution at 0-3^0C to a solution of the coupling component in acetic acid, good yields of dye were usually obtained. To complete the coupling, particularly when nitrosylsulphuric acid was used in the previous diazotization, the pH of the reaction mixture was adjusted to approximately 4-5. Thus, an appropriate amount of 10% sodium acetate solution was slowly added below 5^0C.

Dyeing method

Dyeing of nylon fabric was carried out using a procedure reported in the literature[4].

Exhaustion study

The percentage dye bath exhaustion of the dyed fabric was calculated by the usual method [5].

Wash fastness test

The wash fastness test was assessed in accordance with IS: 765-1979[5].

General

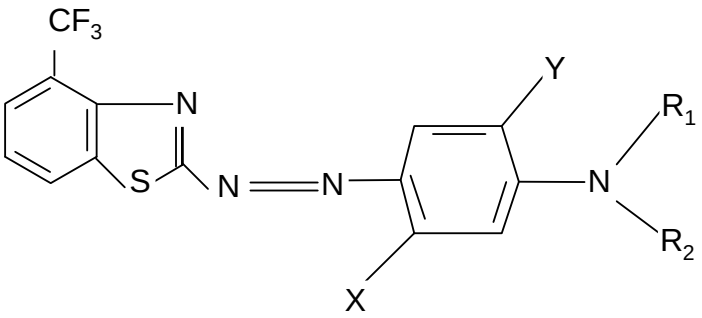
Melting points were determined by the open capillary method. The visible absorption spectra were measured using a Spectro UV-Vis Dual Beam 8 auto cell UVS-2700[6].

3. Results and Discussion

Synthesis of Dyes

Fig. 1 shows the structures of the seven dyes synthesized. All the dyes were synthesized by diazotizing 2-amino-4-trifluoromethylbenzothiazole using the general method of diazotization of weakly basic amines, and coupled to seven different coupling components namely; N,N-dimethylaniline, N,N-diethylaniline, N,N-bis(2-cyanoethyl)aniline, 3-acetyamino-N,N-dethylaniline, N,N-diethylaniline, 5-acetylamino-2-methoxy-N,N-diethylaniline, 2-naphthol, and 1-naphthol respectively. The weakly basic amines contain some electron withdrawing groups which make them insoluble in HCI but are soluble in nitrosyl

sulphuric acid, (formed by the dissolution of sodium nitrite in sulphuric acid). The different dyes synthesized possess distinct characteristics as shown in Fig.1. The structures of the dyes synthesized are planar, thus, can lie flat against the polymer molecules. Their planarity accounts for their good substantivety for hydrophobic fibres[7].



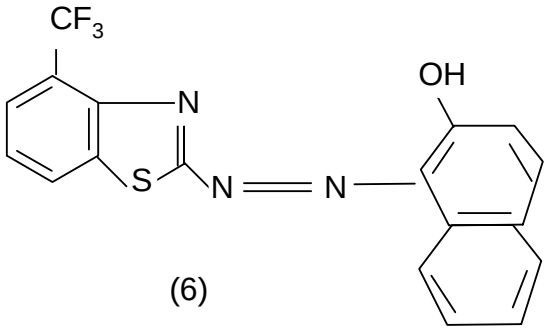
(1), $R_1 = R_2 = CH_3$, X = Y = H

(2), $R_1 = R_2 = C_2H_5$, X = Y = H

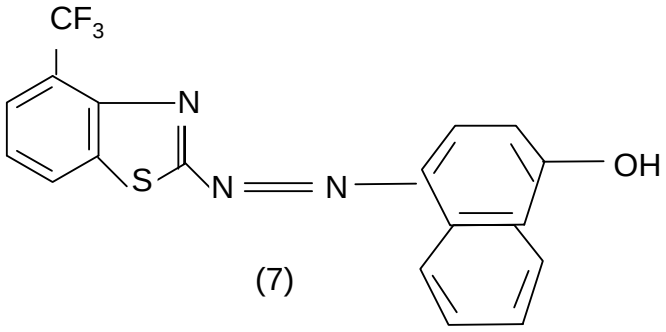
(3), $R_1 = R_2 = C_2H_4CH$, X = Y = H

(4), $R_1 = R_2 = C_2H_5$, X = $NHCOCH_3$, Y = H

(5), $R_1 = R_2 = C_2H_5$, X = $NHCOCH_3$, Y = OCH_3



(6)



(7)

Fig. 1: STRUCTURES OF DYES SYNTHESISED

Spectroscopic properties of the Dyes

The physical characteristics of the dyes are shown in Table 1. The visible absorption spectra of the synthesized dyes in different solvents are summarized in Table 2. Dye (1) which was obtained by coupling the diazo component with N,N-dimethylaniline absorbed at 450 nm in acetone and when the N,N-dimethlyaniline was replaced with N,N-diethlyaniline to give dye (2), the maximum absorption wavelength obtained was 455 nm in the same solvent. This results in a red shift of 5 nm. When two of the hydrogen atoms of the ethyl group in dye (2) were replaced by cyano group to give dye (3), the resulting dye showed maximum absorption wavelength of 466 nm which is more bathochromic than dyes (1) and (2). This was however unexpected since the cyano group is an electron withdrawing group incorporated in the donor ring of the coupler.

Table 1: Physical Characteristics of the Dyes

Dye Number	Melting Point (^oC)	Yield (g)	%Yield	Molecular Wt (g/mol)
1	224	1.08	74	378
2	202	0.90	66	350
3	105	0.81	49	428
4	173	0.51	30	430
5	138	0.45	25	465
6	131	0.50	36	373
7	104	1.34	93	373

Table 2: Spectroscopic Properties of the Dyes

Dye Number	Acetone (λ_{max}) nm	Ethanol (λ_{max}) nm	Ethanol + HCI (λ_{max}) nm	$\Delta(\lambda_{max})$ ethanol-(HCI/ ethanol)	Extinction coefficient (€) $1mol^{-1}cm^{-1}$
1	450	525	535	+10	40,000
2	455	510	515	+5	28700
3	466	485	540	+55	47100
4	457	485	490	+5	38,200
5	486	560	570	+10	38,000
6	482	486	492	+6	36,600
7	484	489	492	+3	38,400

The introduction of acetamino group into dye (2) gave dye (4) with maximum absorption wavelength of 457 nm which is slightly more bathochromic by 2 nm when compared with dye (2) and 7nm when compared with dye (1). Increasing the number of electron donating groups by the introduction of methoxy group into dye (4) gave dye (5) with maximum absorption wavelength of 486 nm in acetone. This gave a red shift of 31nm when compared with dye (2) and 17 nm when compared dye (4). This is the most bathochromic of all the dyes synthesized. This clearly demonstrated that increasing the number of electron donating groups in the coupler ring would produce dyes with enhanced bathochromic shift in the visible absorption region of the spectrum.

Dye (6) was produced by coupling with 2-naphthol, and gave maximum absorption wavelength of 482 nm in acetone. This showed that increasing the conjugation of the dye by using naphthalene ring instead of benzene ring led to the production of highly bathochromic dyes. Changing the hydroxyl group from ortho position to the para-position as shown in dye (7) by using 1-naphthol as the coupling component instead of 2-naphthol gave a red shift of 2nm when compared with dye 6. This was however surprising since dye (6) with the hydroxyl group ortho to the azo chromophoric group was expected to be hydrogen bonded and therefore supposed to be more bathochromic than dye (7) in the same solvent.

When the solvent polarity was changed from acetone to more polar ethanol, all the dyes showed red shifts i.e. positive solvatochromism. For example, dye (1) absorbed at 450 nm in acetone and 525 nm in ethanol, showing a positive solvatochromism of 75 nm. Similarly, dye (5) showed a positive solvatochromism of 7nm. This red shift indicates that the excited states of the dyes are more polarized than the ground state and therefore polar solvents stabilized them more in the excited states. It can also be seen from the results summarized in Table 2 that all the dyes exhibited positive halochromism when a few drops of HCI was added to their ethanolic solutions. For example, dye (1) showed a red shift of 10 nm on addition of one drop of HCI and dye (3) showed a positive halochromism of 55 nm, which is the highest of all the dyes produced.

Effect of Time on Dye Exhaustion

Fig. 2 shows a progression in the percentage exhaustion with increase in dyeing indicating that the longer the dyeing time, the greater the amount of dye molecules absorbed by the fibre, until an equilibrium is attained more dyes penetrate into the polymer at longer dyeing time resulting in high percentage exhaustion and deeper shade. Dye (3) gave a higher exhaustion at the boil for 30 min when compared to dyes (5) which has relatively higher molecular weight, 428 and 465 g/mol respectively. The naphthol dyes, dyes (6) and (7) have the same molecular weight, 373 g/mol each, but their percentage exhaustion at the boil for 30 min varied. Dye (7) gave higher % exhaustion than dye (6) because it is more planar. The ortho position of the hydroxyl group in dye (6) reduces its planarity as compared to the para position in Dye (7).

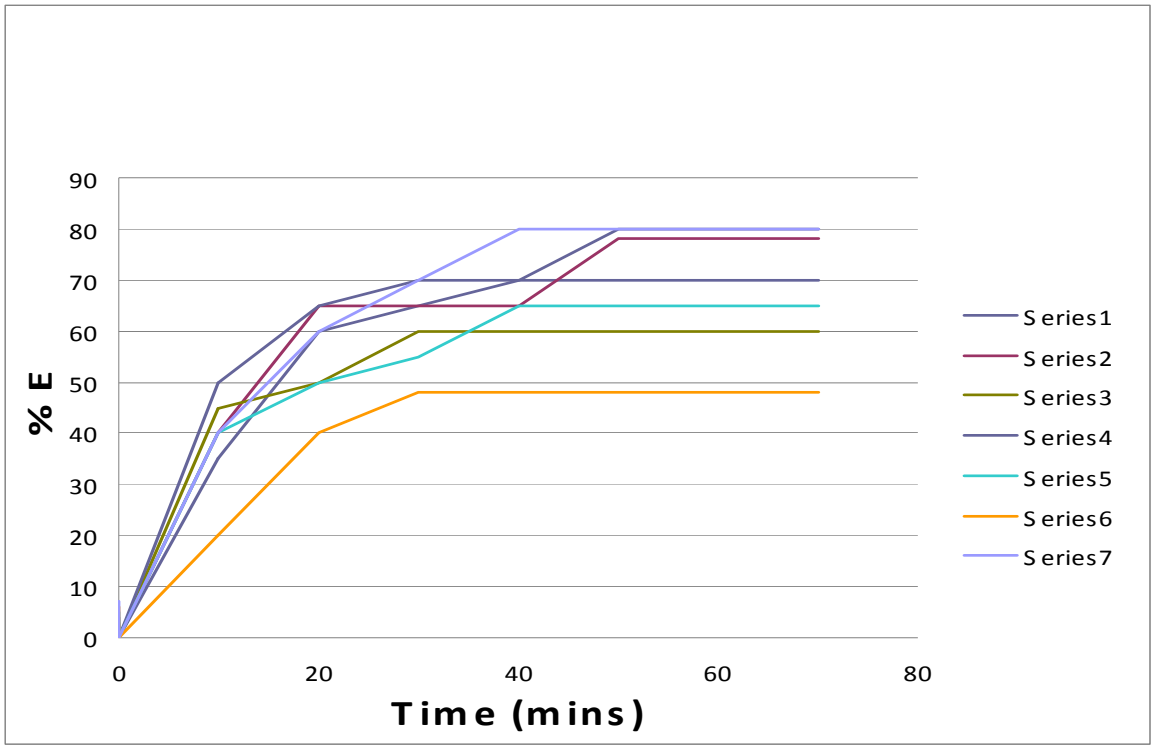


Fig 2: Percentage Exhaustion (% E) at 50^0C

It was noted at the course of this research work that all the dyes synthesized showed higher % exhaustion at the longer dyeing time, but it should be however noted, that longer dyeing time will be undesirable in practice as it will increase the cost of fuel and labour cost, and may also lead to damage of the fibre, as a result of prolonged period of contact with the hot dye-liquor[10]. Thus it will be profitable to adopt effective means of control of the rate of dyeing using disperse dyes.

Effect of Temperature on Dyeing

It is evidently clear from the results illustrated graphically in Fig. 2-4 that temperature change affects dyeing and the percentage dye exhaustion. All the dyes synthesized and applied to polyamide fibre showed high exhaustion at temperatures near the boil or at the boil. This is so, because, there is greater segmental mobility of the fibre polymer chains at higher temperatures and this cases penetration of dye molecules into the fibre[8]. At 100^0C the % exhaustion of the dyes are 85%, 91%, 74%, 93%, 86%, 57% and 93% respectively for dyes (1) to (7). Dyes (4) and (7) show highest % exhaustion while dye (6) gave the lowest percentage exhaustion of 57% at the boil. In dyeing of polyamides fibres with disperse dyes, temperature control is very essential because, it was noted that while some of the dyed samples showed good levelness at the boil, in some few others there was a little degree of unlevelness.

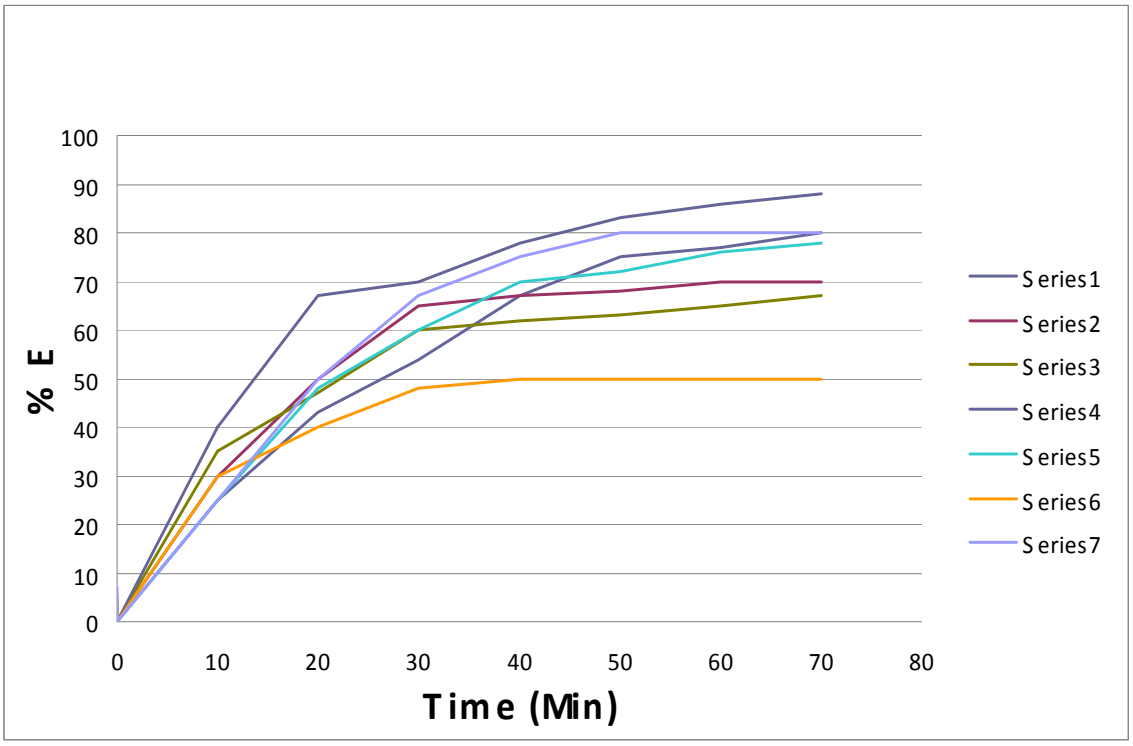


Fig 3: Percentage Exhaustion (% E) at 70^0C

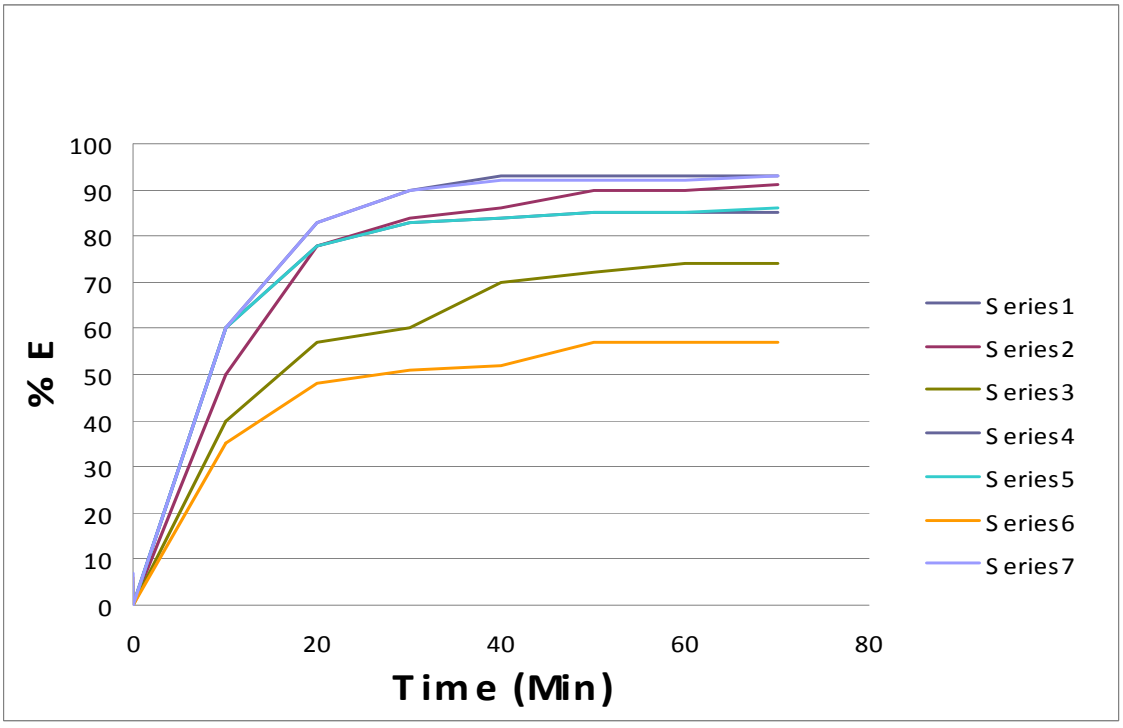


Fig. 4: Percentage Exhaustion (% E) at 100^0C

Wash Fastness Rating of the Dyes

Table 3 shows the I.S.O 3 wash fastness rating of the dyes synthesized. The results show that all the dyes have very good to excellent wash fastness with corresponding little staining, indicating that all the rating values lies between 4 and 5. The excellent wash fastness of the dyes is due to their hydrophobic character and their polarity, conferred on them by the polar substituent groups. The polar substituent groups on the dye molecules contribute greatly to the strength of the dye-fibre bond. The presence of a donor group on the benzothiazole ring also tends to improve their fastness characteristics. The dyes will also exhibit high light fastness on polyster and nylon and will be useful for transfer printing because the trifluoromethyl group on the diazonium component gives the good thermal transfer characteristics necessary for the sublimation process.

Table 3: Wash Fastness rating of the Dyes

Dyes	Colour Change	Staining of adjacent white
1	4	4
2	4	4
3	4-5	4-5
4	4	3-4
5	4-5	4-5
6	4	4
7	4-5	4

Table 4: Thermodynamic Parameters of the Dyes (1-7) on Polyester

Dye	Equilibrium exhaustion (%E)			Partition Coefficient (K)/ℓtkg^{-1}			Standard Affinity ($\Delta\mu^0$ kJmol^{-1})			$\Delta H^0$of Dyeing kJmol^{-1}
	50^{0}C	70^{0}C	100^{0}C	50^{0}C	70^{0}C	100^{O}C	50^{0}C	70^{0}C	100^{O}C	
1	70	80	85	116.7	180	283	12.78	14.81	17.51	-11.945
2	78	70	91	177.3	300	505	13.91	16.2	19.31	-13.713
3	60	67	74	75.0	100	142.0	11.59	13.13	15.38	-9.571
4	80	88	93	200.0	380	664	14.23	16.94	20.15	-14.95
5	65	78	86	92.9	180	307	12.16	14.81	17.76	-14.142
6	48	50	57	46.1	50	66.2	10.29	11.15	13.01	-6.869
7	80	88	93	200	375	664.3	14.23	16.89	20.60	-16.086

The thermodynamic parameters values are shown in Table 4. A comparison of the standard affinity of Dye 1 and Dye 2 showed an increase in value of ($\Delta\mu$) 1.8kJmol^{-1}**.** This showed that alkyl groups increase from CH_3 to C_2H_5 (methylene group CH_2) causes increase in $\Delta\mu$ of 1.8kJmol^{-1}. This is in line with the finding of Meggy and Bello [9, 10]. These results also agreed with the finding of previous workers[11]. On the other hand, a comparison between Dye 2 and Dye 5 showed a decrease of ($\Delta\mu^o$) 1.55kJmol^{-1}. This is caused by the OCH_3 and $NHCOCH_3$ in the ortho and meta position respectively. The substituents in the ortho and meta positions are election withdrawing and therefore reduce, the election cloud on nitrogen, despite the fact that C_2H_5 groups are election releasing.

Comparison of Dyes 4 and 5 is even more interesting, Dye 4 has equilibrium exhaustion %E of 93% while Dye 5 has 84%. The corresponding standard Affinities for Dye 4 and 5 are respectively 20.15 kJmol^{-1} and 17.76 kJmol^{-1} respectively at 100^{0}C. The decrease is $\Delta\mu$ of 2.39 kJmol^{-1} is caused by the presence of OCH_3 in the ortho position in Dye 5 instead H as in Dye 4. For Dyes 6 and 7, the position of the OH seemed to be a major factor in equilibrium exhaustion of the Dye on fabric (polyester) Dye 7 is more polar than dye 6. There seems to be a similarity between results of spectroscopic studied for these dyes and their thermodynamics parameter values.

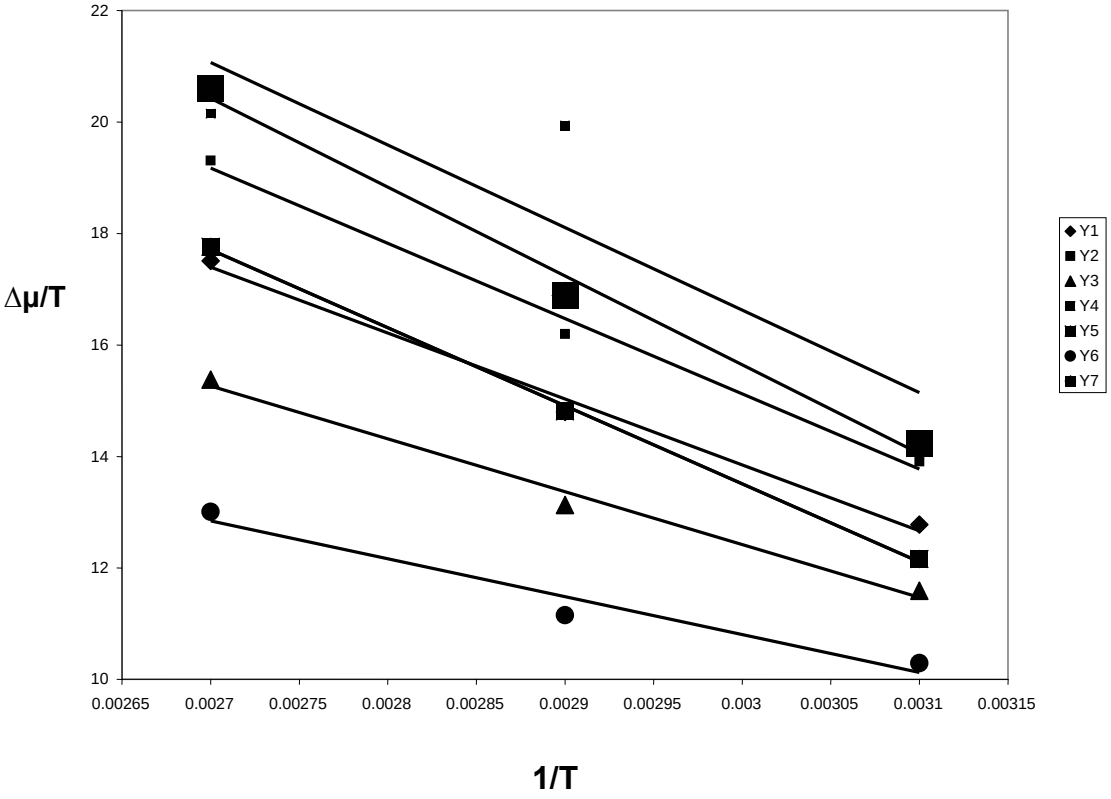


Figure 5. Plot of Δμ/T versus 1/T for dyes 1- 7

4. Conclusion

Monoazo disperse dyes containing N, N-dialkylaniline and naphthol coupling moieties have been prepared from 2-amino-4-triflоromethylbenzothiazole. The dyeing and fastness properties of these dyes have been examined and found to be excellent. A gamut of colour shades ranging from red to blue was obtained by applying the variously substituted dyes on nylon. Bathochromic colours were observed by increasing the numbers of electron donating substitutes and also increasing the conjugation of the coupler ring. The dyes provided a wide range of deep and bright shades on nylon. These dyes gave good exhaustion and level dyeing.

The thermodynamic parameters values of the Dye were determined and the standard affinity ($\Delta\mu^0$) values ranged between 15.38 and 19.31 $kJmol^{-1}$ and the showed that the exhaustion of a particular Dye increases with increase in methyle group (CH_2) of the substituent. The results also showed that decrease in the equilibrium exhaustion and standard affinity decrease with introduction of electron withdrawing groups such as OCH_3.

For dyes 6, and 7, the standard affinities, at 100^0C are 13.0 and 20.6 $kJmol^{-1}$ and the equilibrium exhaustions are 57% and 93% respectively. The pattern of the results of spectroscopic study and the thermodynamic parameter values for these dyes followed the same explanations. In other words the thermodynamic values of these dyes could serve as models for predicting the spectroscopic values of other similar dyes in the series.

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9/1/2009

Phytochemical and Antimicrobial Properties of Four Herbs from Edo State, Nigeria.

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Abstract: Phytochemical and antimicrobial properties of four medicinal plants (*Bidens pilosa, Euphorbia heterophylla, Euphorbia hirta* and *Phyllanthus amarus*) used in the management of some diseases in Edo State, Nigeria were investigated in this study. The results revealed that tannins, saponins, flavonoids, cardiac glycosides, alkaloids and steroids were all present in *E. hirta*. While *P. amarus* contain all except cardiac glycosides. *E. heterophylla* contained saponins, flavonoids, cardiac glycosides and steroids whereas *B pilosa* contained saponins, alkaloids and steroids only. These substances are known components of medicial plants and may explain the use of the preparations of the herbs under study for managing a number of common ailments including dysentery, diabetes, hypertension and some microbial infections among the indigenous communities in Edo State. When tested against *Escherichia coli, Streptococcus spp, Klebsiella spp, Pseudomonas spp* and *Staphylococcus spp,* aqueous and methanolic extracts of *E. hirta, P. amarus* and *B. pilosa,* showed varying degrees of inhibition to the growth of tested organisms. *E. heterophylla,* was effective as an antimicrobial agent only against *Streptococcus sp.* Extracts from all the plants tested had no effects on *Klebsiella sp* used is this study. Methanolic extracts of the plants were more effective than aqueous extracts in inhibiting the growth of the pathogenic bacteria under study, but were less potent when compared to that of ofloxacin and ciprofloxacin used as positive controls. Presence of Phytochemical agents and antimicrobial properties in the tested plant species is confirmed. [Report and Opinion. 2009; 1(5):67-73]. (ISSN 1553-9873)

Key Words: Phytochemicals, antimicrobials, herbal medicine, Edo State.

Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Nostro *et al*., 2000; Tanaka, 2002). Traditional medicine is an important part of African cultures and local medicinal systems vary between different cultural groups and regions (Makhubu 2006). Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficacy and quality assurance of ethno- medicine. In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with good antibacterial activity will be used for the treatment of bacterial infections. This is because, according to Arora and Keur *(*1999), the success story of chemotherapy lies in the continuous search of new drugs to counter the challenges posed by resistant strains of micro organisms. Studies indicate that in some plants there are many substances such as peptides, tannins, alkaloids, essential oils, phenols, and flavonoids among others which could serve as sources for antimicrobial production. These substances or compounds have potentially significant therapeutic application against human pathogens including bacteria, fungi and viruses (Arora and Keur, 1999; Okigbo and Omodamiro 2006).

The development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano *et al.* 1996; Hammer *et al*., 1999). Antibiotic resistance has become a global concern (Westh *et al* 2004) as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug-resistant pathogens (Bandow *et al,* 2003). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for the development of novel drugs because of the great diversity in their

chemical structure. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rogas *et al*., 2003). Therefore, researchers are increasingly turning their attention to ethno-medicine, looking for new leads to develop more effective drugs against microbial infections (Benkeblia, 2004); and this has led to the screening of several medicinal plants for potential antimicrobial activity (Colombo and Bosisio, 1996; Iwu *et al*. 1999).

In the present study, four herbal plants (*Bidens pilosa, Euphorbia heterophylla, Euphorbia hirta* and *Phyllanthus amarus*) which are used in the health care system of Edo State to manage some common ailments and infections were investigated. *Euphorbia* is one of the most diverse genera in the family Euphorbiaceae. Members of the family and genus are sometime referred to as spurgs. The leaves of *Euphorbia heterophylla* are commonly used as a lactogenic agent by taking a decoction of it or massaging the breast with the poultice to induce milk flow (Dokosi 1998). They are also used in traditional medical practice as laxative and to treat gonorrhea, migraine and viral warts while the plant latex is used as fish poison, insecticide and ordeal poisons (Rodriguez *et al.*, 1976, Falodun *et al*, 2003). Recently their anti-tumor/anticancer properties and their activities against the Human Immunodeficiency Virus (HIV) have also been reported in *E. heterophylla* leaf (Williams *et al*., 1995).

Euphorbia hirta is also a member of the Euphorbiaceae family and its common names are Australian asthma plant, garden spurge and snake weed. A decoction of the leaves is taken to induce the flow of milk and the leaf can be chewed with palm kernel for the restoration of virility. A poultice of the leaves is externally applied to abscesses to bring it to a head and for faster pain relief. Similar uses of the plant have been reported in Ghana by Dokosi (1998)

The genus *Phyllanthus* also belongs to the family Euphorbiaceae. The most common species of this genus in Nigeria and most West African nations are *Phyllanthus amarus* and *Phyllanthus niruri*. The leaves of *Phyllanthus amarus* are made into a paste and licked to expel intestinal worms. The decoction of the plant is also used as a purgative. Topically, this plant has soothing effect on the skin and is used for genital and anal infections. Poultice is used for the treatment of certain types of ulcers or sores. The infusion of the root and leaf is a very good tonic and causes diuresis when taken in repeated doses (Oliver 1986). According to Sirajudeen *et al* (2006), administration of *P. amarus* extract is non-toxic with no signs of toxicity or mortality in experimental rats.

Bidens pilosa belongs to the family of *Asteraceae.* The plant is s used for angina, diabetes, dysentery, dysmenorrhea, edema, hepatitis, and laryngitis. In Peru, the leaf is used to treat toothache and headache (Dimo *et al.*, 1999; Rogas *et al.*, 2004). *B. pilosa* has been the subject of recent clinical studies which has explained many of its uses in herbal medicine (Dimo *et al*.2001, Dimo *et al* 2002, Khan *et al* 2001). The selected microbes for this work were *Escherichia coli, Streptococcus, Klebsiella, Pseudomonas and Staphylococcus* spp. which are among the most common bacterial isolates from human infections.(Brown and Anicelus, 2004 ; Donaldson and Gosbell, 2006) .

Aims and Objectives: The objective of this study is to carry out an investigation on four medicinal plants *(E. hirta, E. heterophylla, B. pilosa and P. amarus)* to document their phytochemical and antimicrobial properties which would throw light on their possible mechanism of action and justify their use as anti-microbial agents.

Materials and Methods

The fresh leaves of *Bidens pilosa, Euphorbia heterophylla, Euphorbia hirta, Phyllanthus amarus* were collected randomly from secondary forests and open fields in Edo State of Nigeria. The leaves were washed under running tap water and dried in air for 24 hours. With the aid of grinder, the leaves of the plants were homogenized to fine powder and stored in airtight containers.

Phytochemical tests

One gram of powder was subjected to qualitative phytochemical tests for alkaloids (Myers Reagent), saponins (chloroform and H_2SO_4 tests), inulin (Molischs Reagent) and tannins (Ferric salt tests) adopting the procedures described by Stephen (1970)[26] and Parekh and Chanda (2007)[27]. Flavonoids were determined by the magnesium ribbon test while steroid was confirmed by the chloroform-acetic anhydride test.

Antimicrobial Activity

Aqueous Extraction

A 10g sample of air dried powder was added to 100ml of distilled water and boiled on slow heat for 2 hours. It was then filtered through 6 layers of muslin cloth and centrifuged at 5000g for 10 minutes. The supernatant was collected. This procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours was pooled together and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved at 121^0 C and at 15 lbs pressure and stored at 4^0 C.

Methanol Extraction

A 10g of air dried powder was taken in 100ml of 90% methanol in a conical flask plugged with cotton wool and then kept on a rotator shaker at 190-220 rpm for 24 hours. After 24hours the supernatant was cooled and the solvent was evaporated to make the final volume one-fourth of the original volume and stored at 4^0C in airtight bottles.

The methanolic and aqueous extracts were then tested against five bacteria species sourced from the Irrua Teaching Hospital, Edo State, Nigeria. The tested organisms are *Escherichia coli*, *Streptococcus spp*, *Klebsiella spp*, *Pseudomonas* spp. and *Staphylococcus aureus*. The antibiotics, ciprofloxacin) and ofloxacin at 5µg (disc potency) were used as positive controls following standard methods.

Determination of Antimicrobial Activity of plant Extracts.

The test organisms and the standard strains were separately inoculated into nutrient broth and incubated at 37^0C for 4-6 hours. Then 0.2ml (10^5 cfu/ml) of the broth culture of the test bacteria were seeded on Mueller-Hinton agar plates and spread evenly.

Four wells of 6mm in diameter were cut on the seeded agar plates using a sterile cork borer. Two of the wells were each filled with 0.05ml of hot- water and ethanol extracts.

The remaining two wells contained hot water and ethanol each and these served as control wells. The plates were incubated at 37^0C for 24hrs after which the zones of inhibition round the wells determined the antibacterial activity of the extracts. Zone diameter was recorded as the differences between extracts and any produced by the respective controls. This procedure was repeated for the plant extracts of the four species under study.

Table1: Phytochemical analysis of screened medicinal plant species

Plant Species	Tannins	Saponins	Flavonoids	Cardiac glycoside	Alkaloids	Steroids
Euphorbia hirta	+	+	+	+	+	+
Euphorbia heterophylla	-	+	+	+	-	+
Bidens pilosa	-	+	-	-	+	+
Phyllanthus amarus	+	+	+	-	+	+

+ =Present
- =Absent

Table 2: Traditional uses of four selected herbs from Edo State of Nigeria

Plants	Traditional uses of selected plants
Euphorbia hirta	Dried leaf as vermifuge. Leaf infusion to increase breast milk flow, and to treat diarrhea/dysentery, splenomegally, asthma and whooping cough.
Euphorbia heterophylla	Extract used to treat ear pain. Poultice induces milk flow. Poultice taken in pap to increase sperm quality.
Phyllanthus amarus	Malaria, Chronic stomach pains, oral or vaginal thrush, alcoholic liver disease, hyperglycaemia, urinary tract infection and venereal disease. Taken in honey as aphrodisiac.
Bidens pilosa	Poultice topically applied to sores, for ear aches and intestinal infections. Infusion taken for coughs and colics. Healing of peptic ulcers. Hot infusion of leaves for conjunctivitis.

TABLE 3: Zones of inhibition (mm) of crude aqueous and alcoholic extracts (100mg/ml) of selected herbs, ciprofloxacin and ofloxacin on five bacteria species.

		Zones of inhibition (mm) of extract on selected microbials				
Plant species	**Extract**	***Streptococcus***	***S. aureus***	***E. coli***	***Klebsiella***	***Pseudomonas***
Euphorbia heterophylla	**Aqueous**	**0**	**0**	**0**	**0**	**0**
	Alcohol	**5**	**0**	**0**	**0**	**0**
Phyllanthus amarus	**Aqueous**	**5**	**10**	**6**	**0**	**7**
	Alcohol	**6**	**6**	**10**	**0**	**11**
Euphorbia hirta	**Aqueous**	**0**	**6**	**8**	**0**	**12**
	Alcohol	**0**	**12**	**6**	**0**	**14**
Bidens pilosa	**Aqueous**	**0**	**0**	**5**	**0**	**0**
	Alcohol	**0**	**0**	**5**	**0**	**0**
Controls	**Ofloxacin**	**0**	**0**	**17**	**30**	**21**
	Ciprofloxacin	**8**	**14**	**19**	**0**	**0**
	Sterile Distilled water	**0**	**0**	**0**	**0**	**0**
	Sterile Distilled water	**0**	**0**	**0**	**0**	**0**

0= No inhibition of growth.

Results and Discussion

Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract and the one that predominates over the others. It may also be used to search for bioactive agents that could be used in the synthesis of very useful drugs (Yakubu *et al.*, 2005). Phytochemical screening of the leaves of the selected plants revealed the presence of saponins, alkaloids, steroids and tannins as the major phytochemical components (Table 1).

In the present study, *Biden pilosa, Euphorbia hirta, Euphorbia heterophylla,* and *Phyllanthus amarus* recorded different phytochemicals of medicinal importance although some of their therapeutic properties are exhibited by more than one plant, hence these plants are used for different ailments in the communities under study (Table 2). The results revealed that tannin was present in *E. hirta* and *P. amarus* but absent in *E. heterophylla* and *B. pilosa*. While saponin was present in all the plants, flavonoids were present in *E. hirta, E. heterophylla* and *P. amarus*. Cardiac glycosides were present in *E. hirta* and *E. heterophylla*, but absent in the other two plants studied (Table 1). Alkaloids, Steriods, and saponins were present in all plants examined while alkaloids were present in all except *E. heterophylla* .

The use of *Euphorbia hirta* can be explained by its high content of various phytochemical agents such as tannins, saponins, alkaloid, cardiac glycosides and steroids as it is used by the indigenes to manage a number of ailments. The dried plant is used as vermifuge. It is also used to increase the production of breast milk, an effect likened to that of both oxytocin and prolactin. Its use in the treatment of diarrhoea/dysentery and application to wounds may be due to its antimicrobial actions (Table 2). Ogueke *et al* (2007) reported that the plant is used in the treatment of sores, boils, wounds and control of dysentery and diarhoea among the Igbo ethnic group in Nigeria. In the current investigations the aqueous as well as the methanolic extracts of this plant exhibited antimicrobial properties, thus confirming previous records that the plant has antibacterial properties on certain bacterial species (Gill, 1992)

Similarly, *Euphorbia heterophylla* contains saponins, cardiac glycosides flavonoids , tanins and steroids. Traditionally the leaf of the plant is used to treat ear pain (otitis media or externa) while the poultice induces milk flow from breast. *Phyllantus amarus* is also an important herb used in Edo State for the management of malaria, chronic stomach pains, and thrush or candidiasis. The three species belonging to the Euphobiaceae family (E. *hirta, E. heterophylla and P. amarus*) are used to manage cardio-vascular related health problems, properties that can be attributed to the high content of cardiac

glycosides which is used in treatment of heart failure. Cardiac glycosides were originally prepared from crude digitalis, a preparation from the dried leaf of the foxglove plant *Digitalis purpurea.* Their blood sugar lowering properties may also not be unrelated.

Bidens pilosa is an important herb which also serves as vegetable in times of need (Dosoki 1998). It contains alkaloids, saponins, and steroids as its major chemical components. Poultice from the leaves is externally applied to sores, and is also used for ear aches and intestinal infections. Leaf infusion is taken for coughs and abdominal colics. *Bidens pilosa* is widely used as an antibiotic (Table 2). The exhibited antibacterial properties of *B. pilosa* can be attributed to the presence of saponins, steriods and alkaloids in the leaves. This agrees with t Tomas-Barberan *et al.*, (1990), who reported that these might have complimented or potentiated the saponins in the antibacterial activities in the plants where both alkaloids and saponins were present. According to Dimoi *et al* (1999)[33] *Bidens pilosa* is used to cure angina, diabetes, dysentery, dysmenorhea, oedema, hepatitis, jaundice, laryngitis and worms in Peru. A decoction of the root is used for treating hepatitis and intestinal worms. In the present study, the crude alcoholic extract of the plant exhibited moderate inhibitory effect on the growth of *E coli,* thus justifying its use by local communities for managing some microbial infections in wounds, and some intestinal bacterial infections.

This antimicrobial study showed that the Gram-negative bacterial strains used (*E. coli and Pseudomonas* spp.) were generally more susceptible to the extracts than gram-positive bacteria strains (*S. aureus* and *Streptococcus* spp). *S. aureus* and *Streptococcus* spp exhibited smaller zones of inhibition at the concentration used compared to *E.coli, and Pseudomonas* which are gram negative species (Table 3). That the leaf extract of three of the herb used in the present study is capable of inhibiting the growth of *S. aureus* and *Streptococcus spp* at all is good news since multi-drug resistant strains of the organism are on the increase in both hospital and community environments in Nigeria against orthodox antibiotics (Lamikanra and Ndep, 1993; Chamber, 2001; Brown and Anicelus, 2004). *S. aureus* is reported to be highly resistant to ampicillin, cephalexin, methicillin and vancomycin; and is also resistant to gentamycin, rifampicin and chloramphenicol (Onanuga *et al*., 2005; Donaldson and Gosbell, 2006). Tannins have been isolated from some medicinal plants (Mitcher *et al.*, 1988; Hasfermaria *et al.*1993). Egwim *et al.* (2000) have earlier demonstrated the presence of tannins in *Euphobia hirta* and opined that it may account for its antimicrobial activity against *Salmonella typhi in vitro.* Tannins may decrease protein quality by decreasing digestibility and palatability. It may equally interfere with absorption of iron and this may explain why herbs with high tannin concentrations are not administered to anaemic individuals and so should be used with caution in protein deficient/ kwashiorkor patients.

Tannin can be toxic to bacteria, filamentous fungi and yeast (Harborne, 1973). *Phyllanthus amarus* root and leaves provides a very good tonic and cause diuresis when taken cold in repeated doses. A poultice of the leaves with salt cures scabies infection and without salt is applied on bruise and wounds (Oliver, 1986). In the present study, the crude extract was observed to inhibit the growth of *E. coli, Streptococcus* and *S. aureus.* However its effects are low when compared with standard antimicrobial agents such as ofloxacin and ciprofloxacin which was used in this study as positive controls (Table 3). However, the present results revealed that the alcoholic extracts were more effective than the aqueous extract in inhibiting the growth of the test microbes. It is quite possible that some of the plants that were ineffective in this study do not possess antibiotic properties. It is also possible that the active chemical constituents were not soluble in methanol or water. The drying process may have caused changes to occur in some of the chemical constituents found in these plants. Thus future research will centre on the effects of different solvents and drying methods on the efficacy of the plant extracts as microbial agents.

Conclusion

The four plants studied in this report contain various amounts of phytochemicals such as tannins, saponins, steroids, flavonoids, and alkaloids which are known for their therapeutic effects. This study has confirmed and justified the use of the herbal preparations amongst the people especially those in the rural communities where the practice has become prevalent owing to easy accessibility to the plant and the relatively low cost of the herbal preparations. It is believed that the plants mentioned and used in this research work could be potential sources of drugs if the active ingredients are identified and adequately characterized.

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The Effects of Inorganic Fertilizer on the Yield of Two Varieties of Cucumber (*Cucumis sativus* L.)

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Abstract: Field experiments were conducted for three years during the rainy seasons of 2006 to 2008 at the Teaching and Research Farm of Ambrose Alli University, Ekpoma (Lat. 6^0 45^1 North and Long. 6^0 08' East and an altitude of 460 meters above sea level). Compound fertilizer (N.P.K.20:10:10) was applied at 0, 100, 200, 300 and 400 kg/ha to two cucumber varieties (Ashley and Palmetto) using a 2 x 5 factorial scheme replicated three times. Data were collected on vegetative traits, yield and yield components of cucumber and statistically analyzed. Results revealed significant differences ($P< 0.05$) among the varieties in terms of vine length, number of branches and leaf area, The growth and yield attributes of cucumber including the vine length, number of leaves per plant, number of branches, leaf area, number of fruits per plant, fruit length, fruit girth, fruit weight per plant, fruit number per plant and total yield per hectare increased significantly ($P< 0.05$) with increase in inorganic fertilizer application up to the highest level.
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Keywords: Cucumber, inorganic fertilizer rates and yield

I. Introduction

Cucumber (*Cucumis sativus* L.) is an ancient vegetable and one of the most important members of the Cucurbitaceae family (Thoa, 1998) that is cultivated for its fruit which is a rich source of minerals and vitamins. The fruit is eaten fresh in salads in accompaniment with other vegetables.
The soils where cucumber is cultivated require moderate to high nutrient levels so as to achieve high yields. Infertile soils result in bitter and misshapen fruits which are often rejected by consumers thereby reducing farmers' income.

Most soils in Edo State as a result of cultivation over time, have suffered nutrient depletion such that high yields can only be attained through the judicious application of inorganic fertilizers. The invention of chemical fertilizers has allowed man to raise soil productivity higher than could be attained by relying on natural recycling process. Farmers in this locality for a long time had relied on natural soil recycling process for fertility restoration but as a result of high population, the fallow periods in many communities have been shortened and also cultivation has been extended for more than two years, hence there is a decline in crop yield. Many studies of various crops have shown significant advantages of applying inorganic fertilizers (Akinride,2006).

Many varieties of cucumber exist with varying shapes and sizes, skin colour and carotene content (Simon, 1992). The variation in the performance of cucumber varieties has been widely documented by many scholars (Axelson *et al*., 1980; Manyvong, 1997), which could be as a result of genetic composition or environmental factors.

The study was therefore carried out to determine the effects of inorganic fertilizer on the performance of two varieties of cucumber.

2. Materials and Methods

The experiment was conducted at the Teaching and Research farm of Ambrose Alli University, Ekpoma on Lat. 6^0 45^1 N and Long. 6^0 08^1 E in a forest - savanna transition zone of Edo State, Nigeria. The area is characterised by a bimodal rainfall pattern which starts in late March and ends in late July while the short rainy season extends from September to late October after a dry spell in August. The soil order is a ultisol and the site is classified locally as kulfo series (Moss, 1957).

The site was left fallow for three years after it was cropped to maize, yam and cassava for two years prior to the establishment of the experiment. A composite soil sample was collected from 0-30 cm depth prior to planting to determine the pH and the nutrient status of the soil. Soil pH was analyzed by 1:2 in H_2O, total N content was determined by Kjeldahl method (Bremner, 1965); available phosphorus was analyzed using the modified method of Walkley and Black (Nelson and Sommers,1982) . The NPK fertilizer was bought from the Edo State Ministry of Agriculture and Natural Resources. Chemical analysis of the soil is presented in Table 1.

The experimental site was cleared of existing vegetation and packing of the debris was carried out before it was marked into plots. Tilling of the soil was carried out by using hoes. Planting was done on

23 April 2007 at a spacing of 75 cm x 75 cm. Two seeds were sown per hole which was later thinned down to one plant per stand three weeks after planting giving a plant population of 17,778 plants per hectare. The three inner rows were considered the net plot and five plants from the net plot were tagged from which the growth and yield parameters were recorded.

Fertilizer was applied at three weeks after planting at the rates of 0, 100, 200, 300 and 400kg/ha^{-1} using the side band method. The field was weeded manually using a hoe. A total of three weedings were carried out for adequate weed control at 3, 5 and 7 weeks after planting. The crops were sprayed three times with lamdacyahalothrin as 'Karate' (insecticide) and benomyl (benlate) fungicide at the rates of 2 litres and 1.5kg/ha respectively at 4, 6 and 8 WAP to protect the plants against insect pests and fungal diseases. Harvesting of the cucumber fruits commenced at six weeks after planting when the fruits had turned deep green in colour. Harvesting was done by handpicking the matured fruits twice weekly.

The parameters recorded were vine length, number of leaves, number of branches, leaf area, yield and yield components. Growth parameters were assessed at 4, 6 and 8 weeks after planting. Cucumber vine length was measured by using a flexible tape rule. Number of leaves was assessed by visual count of the green leaves. At every harvest the fruit girth was assessed by using a vernier calliper, the fruit length was measured by using a flexible tape before the fruits was weighed using a 10kg scale. The cumulative weights of the entire harvests (10 times) were summed up for data analysis.

Table 1. Chemical analysis of soil sample at the experimental site.

Properties	Value
pH (in 2: 1 H_2O)	6.18
Organic matter content (g/kg)	20.90
Organic carbon (g/kg)	12.16
Nitrogen (%)	0.06
Exchangeable Ca (cmol/kg)	8.80
Exchangeable Mg (cmol/kg)	0.96
Available P (mg/kg)	15.59
Exchangeable K (cmol/kg)	1.14

3. Results

The vine length of two cucumber varieties as affected by varying rate of inorganic fertilizer at 4, 6 and 8 WAP are shown in Table 2. At 4 WAP, the vine length ranged from 16.65 to 18.07cm and any increase in the fertilizer rate from 0 - 400kg/ha resulted in significant increase in length of vines. Both varieties produced the longest vine at 300 and 400 kg^{-}ha^{-1} fertilizer rate, which was significantly different from the other rates. The control produced the least length of vine, which was significantly different from the 100 kgha^{-1} fertilizer rate ($P<0.05$). At 6 WAP, the vine length ranged from 54.89 to 130.35cm. The longest vines were produced by the Palmetto variety at 400 kgha^{-1} and the control treatments of the two varieties produced the least vines.

At 8 WAP, the vine length of the two cucumber varieties ranged from 152.10 to 277.10cm. Any increase in the fertilizer rate led to significant ($P<0.05$) increase in the vine length in both varieties. The Palmetto variety had the longest vines at the fertilizer rate of 400 kg/ha and the control treatments had the least.

The number of branches of two cucumber varieties as affected by varying inorganic fertilizer rate at 4, 6 and 8 WAP is presented in Table 2. At 4 WAP, the number of branches ranged from 0.81 to 1.28. There were significant differences between the treatment means ($P<0.05$) though the branches formed were few. Increase in the fertilizer rate resulted in subsequent increase in the number of branches in both varieties. At 6 WAP, the number of branches ranged from 4.38 to 11.10 and the differences between the treatments were significant ($P<0.05$). The highest number of branches was recorded in the Ashley variety at 400 kgha^{-1}, which was only slightly different from the Palmetto variety at the same fertilizer rate. The least number of branches was recorded in the control treatments. At 8 WAP, the number of branches ranged from 5.90 to 11.87. There was increase in number of branches with increase in NPK application in both varieties. The highest number of branches was produced by the Ashley variety at 400 kgha^{-1} and the lowest was in the control treatments. The

differences between the treatment means were significant (P<0.05).

The number of leaves of two cucumber varieties as affected by inorganic fertilizer rate at 4, 6 and 8 WAP are shown in Table 2. At 4 WAP, the number of leaves ranged from 5.67 to 7.50. The highest number of leaves was observed in the Palmetto variety at 400 $kgha^{-1}$ fertilizer rate which was significantly different (P<0.05) from the other fertilizer rates. At 6 WAP, the number of leaves ranged from 16.20 to 16.54. The differences between the treatment means were significant (P<0.05). The highest number of leaves was observed in the Palmetto variety at the 400 $kgha^{-1}$ fertilizer rate, which was significantly different from the other variety at the same level of fertilizer application. The number of leaves at 8 WAP ranged from 28.52 to 46.95. The highest number of leaves was produced by the Ashley variety at 400 $kgha^{-1}$ fertilizer rate and the lowest by the control treatments. There were no significant differences between the two varieties.

The mean leaf area of the two cucumber varieties as affected by varying rates of inorganic fertilizer at 4, 6 and 8 WAP are shown in Table 2. At 4 WAP, the leaf area ranged from 856.22 to 1236.36 cm^2. Leaf area increased with increase in fertilizer application up to the highest level. The differences between the treatment means were significant (P<0.05). At 6 WAP, the mean leaf area ranged from 3102.20 to 4199.21 cm^2. The highest leaf area was observed in both varieties at 400 $kgha^{-1}$ fertilizer rate. There was a trend as each successive unit of inorganic fertilizer applied resulted in a corresponding increase in the leaf area. The untreated plots for both varieties produced the least leaf area. There were significant differences between the treatments (P<0.05).The leaf area at 8 WAP ranged from 3762.79 to 4987.56 cm^2. At this stage of cucumber growth, there were significant differences between the treatment means (P<0.05). The highest leaf area was observed in the Ashley variety at the 400 $kgha^{-1}$ fertilizer rate with the Palmetto variety having similar value and the control of Palmetto variety, the lowest. Any increase in the fertilizer resulted in an increase in the leaf area.

Fertilizer application had no significant effect on length and girth of cucumber fruits (Table 3). The number of fruits increased significantly (P<0.05) with increase in NPK application (Table 3). Ashley variety responded more to fertilizer application than Palmetto variety. Fruit number was more than double at the highest rate of NPK application in both varieties.

There was an increase in fruit weight of the Ashley variety with increase in NPK application but the pattern was not so clear in the Palmetto variety (Table 3). There was significant (P<0.05) increase in yield in the two varieties when NPK application was increased up to the highest level (Table 3). Yield was more than double with the application rates of 300 and 400 kg/ha of NPK. The varietal means were however not significantly different.

4. Discussion

The result of this study showed significant increase in some vegetative traits such as vine length, number of leaves, leaf area, and number of branches with increase in the rates of fertilizer application for the two cucumber varieties. Diaz *et al*. (1973), Pandey *et al*. (1974), Bradley *et al*. (1976), Kmiecik (1976), Yuasa and Aboaba (1981), El-Badawi (1994), Lawal (2000) and Agba and Enya (2005) had all reported increase in growth and yield components of cucumber to applied fertilizer. The improved supply of plant nutrients to cucumber by the application of fertilizer would led to better utilization of carbon and subsequent synthesis of assimilates (Lawal, 2000). Grubben (1997) also obtained good vegetative growth in cucumber due to the application of fertilizer in northern Nigeria. Similarly, Ibrahim *et al.* (1997) reported increase in vegetative growth in watermelon treated with fertilizer. Increasing the rate of NPK fertilizer resulted in an increase in dry matter accumulation per plant. The dry matter weight of cucumber increased with increase in the rate of fertilizer application. This increase was in conformity with the findings of Lamido (1994). El-Badawi (1994) also reported significant increase in cucumber growth and yield with increasing fertilizer levels up to 75 kgN/ha in Samaru Zaria.

The significant response of parameters evaluated (leaf area, vine length, number of branches, number of fruits per plant, weight of fruits per plant, fruit length and girth and yield) to applied NPK fertilizer may be an indication that the nutrients taken up by the plant were well utilized in cell multiplication, amino acid synthesis and energy formation hence increase in photosynthesis. The products of photosynthesis were then translocated to the sinks (fruits and growing buds).This was in consonance with the findings of of El-Badawi (1994) and Lawal (2000) who reported significant response of cucumber fruit weight per plant and total yield to applied inorganic fertilizer.

The cucumber vegetative characters such as vine length, number of leaves, number of branches and leaf area responded significantly to applied inorganic fertilizer up to the 400 kg/ha .This

resulted in the development of the crop and its photosynthetic apparatus and therefore enhancing assimilate production and accumulation. The assimilates produced during photosynthesis were translocated to the various sinks which resulted in the increase in the number of fruits per plant and total yield. The result of this study is also in agreement with the findings of Ogunremi (1990) who reported increase in the yield of melon fruits due to fertilizer application. The number of female flowers in all fertilizer treatments showed significantly higher mean number than the control. From the study, there is the need for effective fertilizer application as infertile soils result in bitter and misshapen fruits which are often rejected by consumers and hence reduction in the farmers earnings.

CONCLUSION

The use of inorganic fertilizer increased the growth and yield attributes of two varieties of cucumber. Increasing the rate of fertilizer up to the highest level of 400 kg/ha gave the highest yield for the two varieties.

Table 2. The effects of inorganic fertilizer application on the vegetative traits of two cucumber varieties evaluated at 4, 6 and 8 WAP

Varieties	NPK (kg/ha)	Vine length (cm) WAP			No of branches/plant WAP			No of leaves/plant WAP			Leaf area (cm^2)/plant WAP		
		4	6	8	4	6	8	4	6	8	4	6	8
Ashley	0	16.65	54.89	152.10	0.81	4.38	5.90	5.77	16.20	28.52	856.22	3102.20	3762.79
	100	17.17	60.90	179.35	0.96	5.20	7.18	6.41	19.33	32.84	950.91	3481.35	4276.59
	200	17.51	75.72	196.01	1.05	7.48	8.73	6.54	21.06	37.04	995.90	3647.85	4400.41
	300	17.90	118.63	229.86	1.20	8.65	10.35	6.77	27.67	40.91	1155.01	3962.14	4670.21
	400	18.07	119.75	274.17	1.28	11.10	11.87	5.77	32.87	45.97	1236.36	4103.12	4987.56
	Mean	17.46	85.90	206.30	1.06	7.35	8.80	6.25	23.61	37.06	1038.67	3659.85	4419.69
Palmetto	0	16.69	55.13	156.47	0.85	4.60	5.70	5.67	16.54	29.49	886.80	3195.49	3464.37
	100	17.20	65.50	185.65	0.98	5.58	6.60	6.43	20.39	32.84	951.69	3507.74	3961.99
	200	17.68	83.27	196.34	1.12	6,63	7.40	6.58	27.07	33.95	999.22	3717.60	4168.01
	300	17.94	113.19	239.99	1.22	8.68	11.03	6.74	33.38	40.66	1080.91	4023.95	4784.16
	400	18.05	130.35	277.10	1.29	10.45	11.05	7.50	36.01	43.65	1170.88	4199.21	4930.92
	Mean	17.51	89.49	208.71	1.09	7.18	8.35	6.58	28.68	36.63	1028.22	3728.82	4340.79
LSD(P<0.05)													
Fertilizer means		0.336	9.486	14.266	0.066	0.797	0.825	0.419	2.464	1.366	42.555	129.366	150.300
Variety means		0.213	5.999	9.023	0.042	0.505	0.523	0.265	1.599	0.864	26.917	81.819	95.058
Interaction means		0.416	13.415	20.175	0.093	1.128	1.165	0.592	3.485	1.931	60.189	182.952	212.556

Table 3. The effects of inorganic fertilizer application on the yield and yield components of two cucumber varieties.

Varieties	NPK (Kg /ha)	Fruit length (cm)	Fruit girth (cm)	Fruit number/ plant	Fruit wt (kg)/plant	Yield (kg/ha)
Ashley	0	16.54	5.13	4.72	1.18	21037.02
	100	16.70	5.02	6.27	1.28	22755.54
	200	17.79	5.25	8.47	2.30	32029.61
	300	16.67	5.01	12.24	2.40	42607.38
	400	16.50	5.01	12.99	2.61	46340.72
	Mean	16.80	5.08	8.94	1.90	33754.05
Palmetto	0	16.83	5.07	4.80	1.15	20385.17
	100	18.06	5.49	6.85	1.29	22933.32
	200	17.63	5.56	8.89	1.14	38044.42
	300	16.63	5.14	12.54	2.46	43792.59
	400	16.93	5.32	12.71	2.57	45748.13
	Mean	17.22	5.31	9.16	1.92	34180.72
LSD(P<0.05)						
Fertilizer means		1.190NS	0.244NS	0.692	0.205	3355.372
Variety means		0.753NS	0.154NS	0.438	0.130	2122.123
Interaction means		1.683NS	0.345NS	0.978	0.290	4745.212

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Effect of Time of Planting on the Growth and Yield of Five Varieties of Cucumber (*Cucumis sativus L.*)

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Abstract: A field experiment was conducted during the wet season of 2006 at the Teaching and Research Farm of the Ambrose Alli University, Ekpoma (Lat. 6º 45' North and Long. 6º 08' E) and 460 meters above sea level to evaluate five varieties of cucumber (Marketmore 76, Ashley, Palmetto, Marketer and Beith Alpha) and determine the appropriate time during the wet season for planting. Planting was done in the month of April, May and June. The results of the study revealed significant differences ($P< 0.05$) among the varieties in terms of vine length, number of branches, leaf area, number of fruit, per plant and total fruit weight per hectare. The highest fruit, yield per hectare was obtained in the April planting and the Ashley variety consistently had higher yields than the other varieties.
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Key words: Cucumber, five varieties, time of planting and yield.

1. Introduction

Cucumber is a major vegetable crop worldwide and develops rapidly, with a shorter time from planting to harvest than for most crops (Wehner and Guner, 2004). The crop is the fourth most important vegetable crop after tomato, cabbage and onion in Asia (Tatlioglu, 1993); the second most important vegetable crop after tomato in Western Europe (Phu, 1997) and is the fourth most cultivated vegetable in the world after tomatoes, brassicas and onions (Wehner, 2007). In tropical Africa, the crop has not been ranked because of limited use.

Cucumber is grown widely in different parts of the world. It is an all year round out door vegetable in the tropics and an important greenhouse vegetable especially in Northern Europe and North America (Mingbao, 1991). Phu (1998) stated that cucumber could be cultivated in the field during the summer and winter in greenhouses using artificial heating. Jizhe (1993) opined that cucumber is a typical vegetable of warm temperate and cool tropical areas that can be cultivated at any time of the year.

At present, cucumber is cultivated as a field crop in most areas of the world under frost free conditions and also an important greenhouse crop in Northern Europe (George, 1990), the United States (Thompson and Kelly, 1957), and China (Jizhe, 1993). Nu (1998) stated that cucumber is a warm season crop which can be cultivated at any time but has little or no tolerance to frost and that growth and development are favoured by temperatures above 20^0C. In Nigeria, cucumber can be cultivated at anytime of the year. During the raining season, the crop is grown under rainfed conditions and during the dry season using irrigation facilities; as a result the crop can be seen in most vegetable markets in Nigeria throughout the year.

Many varieties of cucumber exist with varying shapes, skin colour and carotene content (Simon,1992) .The variation in the performance of cucumber varieties has been widely documented by many scholars (Manyvong, 1997; Ajisefinanni, 2004), which could be as a result of environmental factors or genetic composition.

This study was carried out to evaluate five varieties of cucumber and determine the appropriate time during the wet season for planting.

2. Materials and Methods

The experiment was conducted at the Teaching and Research farm of Ambrose Alli University, Ekpoma on Lat. 6^0 45^1 N and Long. 6^0 08^1 E in a forest - savanna transition zone of Edo State, Nigeria. The area is characterized by a bimodal rainfall pattern which starts in late March and ends in late July while the short rainy season extends from September to late October after a dry spell in August and the rainfall data is presented in Table 1. The soil order is an ultisol and the site is classified locally as kulfo series (Moss, 1957).

The site was left fallow for three years after it was cropped to maize, yam and cassava for two years prior to the establishment of the experiment. A composite soil sample was collected from 0-30 cm depth prior to planting to determine the pH and the nutrient status of the soil. Soil pH was analyzed by 1:2 in H_2O, total N content was determined by

Kjeldahl method (Bremner, 1965); available phosphorus was analyzed using the modified method of Walkley and Black (Nelson and Sommers, 1982) . The NPK fertilizer was bought from the Edo State Ministry of Agriculture and Natural Resources. Chemical analysis of the soil is presented in Table 2.

The experimental site was cleared of existing vegetation and packing of the debris was carried out before it was marked into plots. Tilling of the soil was carried out by using hoes. Planting was done on 1st of April, 1st of May and 1st of June 2006 at a spacing of 75 cm x 75 cm. Two seeds were sown per hole which were later thinned down to one plant per stand three weeks after planting giving a plant population of 17,778 plants per hectare. The three inner rows were considered the net plot and five plants from the net plot were tagged from which the growth and yield parameters were recorded.

Fertilizer was applied at three weeks after planting at the rate of 200 kg/ha^{-1} using the side band method. The field was weeded manually using a hoe. A total of three weedings were carried out for adequate weed control at 3, 5 and 7 weeks after planting. The crops were sprayed three times with lamdacyahalothrin as Karate (insecticide) and benomyl (benlate) fungicide at the rates of 2 litres and 1.5kg/ha respectively at 4, 6 and 8 WAP to protect the plants against insect pests and fungal diseases. Harvesting of the cucumber fruits commenced at six weeks after planting when the fruits had turned deep green in colour. Harvesting was done by handpicking the mature fruits twice weekly.

The parameters recorded were vine length, number of leaves, number of branches, leaf area, yield and yield components. Cucumber vine length was measured by using a flexible tape rule. Number of leaves and number of branches were assessed by visual count of the green leaves and branches and the leaf area was assessed by the dry weight method of Bloodworth and Rhoads, At every harvest, the fruit girth was assessed by using a vernier calliper, the fruit length was measured by using a flexible tape before the fruits were weighed using a 10kg scale. The cumulative weights of the entire harvests (10 times) were summed up for data analysis.

Table 1. Rainfall data taken during the experimental period

Months	No of raining days	Rainfall (mm)
January	2	5.30
February	2	9.20
March	6	96.90
April	5	112.00
May	13	234.10
June	15	292.70
July	19	449.90
August	15	348.10
September	25	554.20
October	19	42.20
November	1	2.20
December	0	0
Total	122	2145.30

Source EADP Ministry of Agriculture and Natural Resources Irrua Edo State.

Table 2. Chemical properties of the soil at the experimental site

Properties	Value
pH (in 2: 1 H_2O)	6.13
Organic matter content (g/kg)	36.50
Organic carbon (g//kg)	21.20
Nitrogen (%)	0. 12
Exchangeable Ca (cmol/kg)	5.36
Exchangeable Mg (cmol/kg)	1.04
Available P (mg/kg)	10.30
Exchangeable K (cmol/kg)	0.15

Results

Vegetative traits

The mean vine length of five cucumber varieties planted in April and assessed at 4, 6 and 8 WAP are shown in Table 3. The mean vine length at 4 WAP ranged from 19.09 to 25.99cm. Ashley and Palmetto varieties had the longest vine length, which was significantly different from the other varieties ($P<0.05$). At 6 WAP, the mean vine length ranged from 144.57 to 179.94cm. Ashley variety produced the longest vines followed by the Palmetto variety. At 8 WAP, the mean vine length ranged from 231.39 to 264.22cm. The longest mean vine length was produced by the Ashley variety, which was significantly similar to the Palmetto variety but significantly different from the other varieties ($P<0.05$).

The mean number of branches at 4WAP ranged from 1.01 to 1.25. (Table 3). The highest number of branches was recorded by the Ashley variety and the lowest by Marketmore 76. The differences between the treatment means were not significant. At 6 WAP, the mean number of branches ranged from 5.84 to 14.40; the highest number of branches was recorded by Ashley variety and the lowest by Marketmore 76 variety (Table 4). The differences between the treatment means were significant ($P<0.05$). At 8 WAP, the mean number of branches ranged from 14.08 to 16.60, the highest number of branches was produced by the Ashley variety and the lowest was by the Super marketer. The difference between the varieties were significant ($P<0.05$).

The mean number of leaves assessed at 4,6 and 8 WAP are presented in Table 3. The mean number of leaves per plant at 4 WAP ranged from 6.26 to 6.96. The highest number of leaves was produced by the Ashley variety and the least by the Marketmore 76 (Table 3). Data on the mean number of leaves per plant at 6 WAP ranged from 43.36 to 58.02. The highest number of leaves was produced by the Ashley variety and the least by the Beith Alpha variety and the differences between the varieties were significant ($P< 0.05$).The mean number of leaves at 8 WAP ranged from 47.84 to 62.49 and the Ashley variety produced the highest number of leaves which was significantly different from the other varieties ($P<0.05$).

The mean leaf area per plant of five cucumber varieties assessed at 4, 6 and 8 WAP are shown in (Table 3). The mean leaf area at 4 WAP ranged from 892.40 to 992.52 cm^2. The highest mean leaf area was observed in the Beith Alpha variety and the lowest mean leaf area was observed in the Marketmore 76. The differences between the varieties were significant ($P<0.05$). The mean leaf area at 6 WAP, ranged from 2971.38 to 4099.23 cm^2. The Ashley variety had the highest leaf area which was significantly different ($P<0.05$) from the other varieties. At the 8 WAP sampling period, the mean leaf area ranged from 3434..58 to 4284.52 cm^2. The highest leaf area was observed in the Ashley variety and the lowest was recorded in the Beith Alpha and the differences between the treatment means were significant ($P<0.05$).

The mean vine length of five cucumber varieties planted in May 2006 is shown in Table 4. The mean vine length at 4 WAP ranged from 23.99 to 28.86cm. The longest vine was observed in the Ashley variety with the Palmetto variety having similar value while the least vine was observed in the Marketmore 76 variety. The differences between the treatment means were significant ($P<0.05$). At 6 WAP, the vine length ranged from 144.99 – 168.45cm. The longest vine was observed in the Ashley variety, and the Beith Alpha the lowest. There was a significant difference in their treatment means ($P<0.05$). The vine length at 8 WAP ranged from 217.43 to 244.02cm. The Ashley variety produced the longest vine and the Beith Ashley, the lowest. The differences between the treatment means was significant ($P<0.05$).

The mean number of branches of five cucumber varieties is presented in Table 4. There were

significant differences (P<0.05) across the three sampling periods of 4, 6 and 8 WAP. At 4 WAP, the mean number of branches ranged from 1.23 to 1.43. The highest number of branches was observed in the Ashley variety with the Palmetto variety having similar values. The Beith Alpha and Marketmore 76 varieties had the least number of branches. The number of branches at 6 WAP ranged from 7.32 to 13.23. The highest number of branches was recorded in the Ashley variety with the Palmetto variety having a similar value. Although the Marketmore 76 variety had the least value, it was similar to the other varieties. At 8 WAP, the number of branches ranged from 10.23 to 14.76. The Ashley variety produced the highest number with the Palmetto variety having a similar value and the Beith Alpha variety, the lowest.

The mean number of leaves of five cucumber varieties at 4, 6 and 8 WAP are shown in Table 4.The mean number of leaves at 4 WAP ranged from 7.75 to 8.22. The highest number of leaves was produced by the Ashley variety and the Marketmore 76, the lowest. The difference between the treatment means was not significant. At 6 WAP, the mean number of leaves ranged from 33.61 to 42.87. The highest number of leaves was produced by the Super marketer variety with Palmetto and Ashley having similar values and Marketmore 76 variety, the lowest. There were significant differences between the treatment means (P<0.05). At 8 WAP sampling period, the mean number of leaves ranged from 39.44 to 48.87. The highest number of leaves was produced by the Super marketer variety with the Palmetto and Ashley varieties having similar values and the Marketmore 76 variety, the lowest. There were significant differences between the treatment means (P<0.05).

The mean leaf area of five cucumber varieties at 4, 6, and 8 WAP are shown in Table 4. At 4 WAP, the mean leaf area per plant ranged from 881.91 to 993.24 cm^2. The highest leaf area was observed in the Ashley variety and the Super marketer, the lowest. There was no significant difference between the treatment means. At 6 WAP, the mean leaf area ranged from 3015.56 to 4227.93cm^2. The highest mean leaf area was observed in the Ashley variety, and the Beith Alpha, the lowest. The differences between the treatment means were significant (P<0.05). At 8 WAP, the mean leaf area ranged from 3759.57 to 4879.70 cm^2. The Ashley variety produced the highest mean leaf area and the Beith Alpha, the lowest. The differences between the treatment means were significant (P<0.05).

The mean vine length of five cucumber varieties planted in June and evaluated at 4, 6 and 8 WAP are shown in Table 5. The mean vine length at 4 WAP ranged from 20.26 to 25.23cm. The Super marketer variety produced the longest vine and the Marketmore 76 variety, the shortest. There were significant differences between the treatment means (P<0.05). At 6 WAP, the mean vine length ranged from 150.82 to 11.03cm. The longest vine was observed in the Super marketer and the Beith Alpha the shortest. There was no significant difference between the treatment means. At 8WAP, the mean vine length ranged from 232.10 to 259.33cm. The longest vine was recorded in the Palmetto variety and the Beith Alpha variety, the lowest; there were significant differences between the treatment means (P<0.05).

The mean number of leaves per plant of cucumber at 4, 6 and 8 WAP is shown in Table 5. There were significant differences between the treatment means at (P<0.05) across the three sampling periods of 4, 6, and 8 WAP. At 4 WAP, the mean number of leaves ranged from 5.24 to 6.13. The highest number of leaves was observed in the Super marketer while the Beith Alpha, the lowest. At 6 WAP, the mean number of leaves ranged from 39.44 to 52.07. The highest number of leaves was recorded in the Palmetto variety and the Beith Alpha, the lowest. At 8 WAP, the mean number of leaves ranged from 45.39 to 56.20. The highest number of leaves was observed in the Palmetto variety and the Beith Alpha, the lowest.

The mean number of branches per plant of five cucumber varieties at 4, 6, and 8 WAP are shown in Table 5. There were significant differences between the treatment means across the three sampling periods of 4, 6 and 8 WAP (P<0.05). At 4 WAP, the mean number of branches ranged from 0.89 to 1.29. The highest number of branches was recorded in the Palmetto variety and the Beith Alpha and Marketmore 76 varieties, the lowest. At 6WAP, the number of branches ranged from 6.22 to 10.14, the highest number of branches was observed in the Super marketer variety and the Marketmore 76 variety, the lowest. At 8 WAP, the mean number of branches ranged from 7.86 to 10.80. The highest number of branches was observed in the Super marketer variety and the Marketmore 76, the lowest.

The mean leaf area per plant (cm^2) of five cucumber varieties of cucumber assessed at 4, 6 and 8 WAP are shown in Table 5. The mean leaf area at 4 WAP ranged from 966.14 to 955.63cm^2. The highest mean leaf area was observed in the Ashley variety with the Palmetto and Super marketer having similar values. The Marketmore 76 variety had the lowest

mean. There were significant differences between their means (P<0.05). The mean leaf area of five cucumber varieties at 6 WAP ranged from 3536.84 to 3757.49 cm^2. The highest leaf area was observed in the Ashley variety and the Marketmore 76 variety, the lowest but there was no significant difference between the treatment means. At 8 WAP, the mean leaf area ranged from 3603.86 to 3961.34cm^2. The highest leaf area was observed in the Palmetto variety and the Beith Alpha variety, the lowest but there was no significant difference between the means.

Yield and yield components.

The fruit girth of five cucumber varieties planted in April are shown in Table 6. The fruit girth ranged from 5.50 to 6.40cm; the highest was observed in the Beith Alpha variety and the Super marketer variety, the lowest. The differences between the varieties were significant (P<0.05).

The fruit length of five cucumber varieties are shown in Table 6. The fruit length ranged from 19.04 to 20.51cm, the longest fruit was observed in the Marketmore 76 variety and the Ashley variety, the shortest but there were however no significant differences between the varieties .

Fruit number per plant of five varieties of cucumber are shown in Table 6.The fruit number per plant ranged from 6.16 to 9.53 and the Ashley variety produced the highest number of fruit per plant and the Marketmore 76, the lowest. There were significant differences between the varieties (P<0.05).

The fruit weight per plant and total yield per hectare of five cucumber varieties are presented in Table 6. The fruit weight per plant ranged from 1.17 to 2.33kg .The Ashley variety produced the highest weight per plant while the Marketmore 76, the lowest but there was no significant difference between the varieties. The total yield of cucumber per hectare ranged from 20,906.85 to 41,098.03kg. The highest yield was observed in the Ashley variety and the Marketmore 76 variety, the lowest but there was no significant difference between the varieties.

The fruit girth, fruit length, fruit weight per plant and yield per hectare of five cucumber varieties planted in May 2006 are shown in Table 7. The fruit girth of five varieties of cucumber ranged from 5.06 to 5.65cm. The Ashley variety had fruits with the widest girth and the Beith Alpha the shortest. The fruit length of five cucumber varieties ranged from 14.64 to 16.59cm. The Ashley variety had the longest fruit while the Beith Alpha, the shortest. There were significant differences between the varieties (P<0.05). The number of fruits per plant of five cucumber varieties are shown in (Table 6). The number of fruit per plant of five cucumber varieties ranged from 5.60 to 8.50; the highest number of fruits per plant was observed in the Ashley variety and the Beith Alpha, the lowest. The differences between the varieties was significant (P<0.05).

The fruit weight per plant and fruit yield per hectare of five cucumber varieties are shown in Table 7. The fruit weight per plant ranged from 1.29 to 2.37kg; the Ashley variety produced the highest fruit weight per plant and the Marketmore 76 variety, the lowest; though there was no significant difference between the varieties. The yield per hectare of five cucumber varieties ranged from 23,431.54 to 29984.31kg; the Ashley variety produced the highest fruit weight per hectare and the Marketmore 76, the lowest. There were significant differences between the varieties (P<0.05).

The yield components of five cucumber varieties planted in June 2006 are shown in Table 8.The fruit number per plant of five varieties of cucumber ranged from 3.84 to 6.66; the highest number of fruits per plant was observed in the Ashley variety and the Marketmore 76, the lowest but there was no significant difference between the varieties.

The mean fruit girth and fruit length of five cucumber varieties are shown in Table 8. The fruit girth ranged from 4.40 to 4.90cm; the widest girth was recorded in the Beith Alpha variety and the Marketmore 76 variety, the shortest but there was no significant difference between the varieties. The fruit length ranged from 15.74 to 17.19cm. The longest fruit was observed in the Palmetto variety and the Ashley variety, the shortest but there was no significant difference between the varieties.

The mean fruit weight per plant and total yield of five varieties of cucumber per hectare are shown in Table 8. The fruit weight per plant ranged from 1.00 to 1.68kg; the Ashley variety produced the highest fruit weight per plant and the Marketmore 76, the lowest. The differences between the varieties were significant (P<0.05). The total yield per hectare ranged from 18,212.70 to 29,892.08kg; the highest total yield per hectare was observed in the Ashley variety and the Marketmore 76 variety, the lowest. There were significant differences between the varieties (P<0.05).

Planting dates and yield of cucumber varieties

The effects of planting dates on the five varieties of cucumber are shown in Table 9. The mean yield of five varieties of cucumber for the three months of April, May and June 2006 were significantly different ($P<0.05$) and ranged from 20,850.71 -33,658.14kg/ha. The Ashley variety produced the highest yield per hectare, and the Marketmore, the lowest. Time of planting also significantly ($P< 0.05$) affected yield; but there was no varieties and planting dates interaction Planting in April produced the highest yield, and the June planting, the lowest..

4 Discussion

The results of this study showed that there were significant differences among varieties in some vegetative characters, namely. Vine length, number of branches, leaf area, number of leaves and yield characters such as number of fruits per plant, fruit weight per plant and total yield per hectare.

In all cases, Ashley and Palmetto varieties were superior to Marketmore 76, Super marketer and Beith Alpha varieties in both the vegetative characters at 8WAP and yield characters. The Ashley variety had differential yield characters – number of fruits per plant, weight of fruit plant and total yield per hectare which were significantly different from the other varieties. These differential growth and yield characters of cucumber have been reported by researchers in different parts of the world. (Sarwar, 1975; Koterowa et al., 1977; Buitelaar, 1978; Haben, 1980; Ramirez *et al*., 1988; Widders and Price, 1989; Uruk,1998). The differences in vegetative and yield characters can be attributed to genetic composition of the varieties used; the Ashley variety may have been quicker in adapting to the environment than the other varieties or the vegetative characters of the Ashley variety may have been more active than the other varieties and therefore had a strong source to sink relationship which resulted in high yields experienced in the variety. The number of fruits per plant was higher than what was reported by Phu (1998) and by Jizhe (1993) in Thailand.

The yields obtained in this varietal studies was higher than what Manyvong (1998) and Phu (1997) reported in Thailand but lower than the yield obtained by Mingbao (1993).

From the result of this study, it was found that cucumber can be planted at any time during the rainy season because of the consistent high yields recorded in the varietal trials which were carried out in different months of April, May and June in 2006. This is in consonance with the findings of Mas (1983) who stated that cucumber can be planted at any time of the year provided during the growing period, there is ample moisture and the soil is fertile. The results also agree with the findings of Thoa (1998) who observed that cucumber can be planted at anytime of the year and that even in temperate regions, during the winter the crop can be grown under greenhouse conditions using artificial lighting systems.

The high yield which was consistently recorded by the Ashley variety throughout the three months of study could be attributed to the genetic composition and its ability to quickly adapt to this environment. This was in agreement with the findings of Staub and Bacher (2004) who posited that cucumber yield is influenced by genetic and environmental factors, and as such is variable depending upon growing season and region.

The high yield experienced during the April planting over the May and June plantings could be attributed to moderate rainfall at the flowering and fruiting stage of the crop which began in the middle of May. High rainfall during flowering and fruiting can lead to bees inactivity with subsequent flower abortion with resultant low yield which may have resulted in the yields experienced during the July planting. This is in agreement with the finding of Papadopoulos (1994), who stated that high moisture tend to discourage the activity of bees and resultant high relative humidity which influence water condensing on the plant leaves which may result in the development of pests and diseases.

Table 3. Vegetative traits of five varieties of cucumber planted in April 2006

	Vine length (cm) WAP			Number of branches/ plant WAP			Number of leaves/plant WAP			Leaf area (cm^2/plant) WAP		
Varieties	**4**	**6**	**8**	**4**	**6**	**8**	**4**	**6**	**8**	**4**	**6**	**8**
Market more 76	22.34^{b}	148.57^{c}	237.86^{c}	1.01	5.84^{a}	14.08^{a}	6.26^{b}	47.30^{b}	53.25^{b}	892.40^{b}	3260.61^{b}	3982.42^{ab}
Ashley	25.99^{a}	174.94^{a}	264.22^{a}	1.25	14.40^{a}	16.60^{a}	6.96^{a}	58.02^{a}	62.49^{a}	979.15^{a}	4099.23^{a}	4284.52^{a}
Palmetto	25.36^{a}	165.54^{a}	250.27^{ab}	1.19	12.00^{b}	15.44^{a}	6.81^{a}	47.95^{b}	53.00^{b}	972.36^{b}	3351.63^{b}	3813.46^{bc}
Super marketer	20.99^{bc}	150.18^{c}	236.13^{b}	1.11	10.08^{c}	13.28^{b}	6.51^{b}	49.33^{b}	53.33^{b}	920.22^{ab}	3235.44^{b}	3443.40^{c}
Beith Alpha	19.09^{c}	152.19^{c}	231.39^{b}	1.07	9.72^{c}	14.08^{a}	6.43^{b}	43.36^{b}	47.84^{b}	992.56^{a}	2971.38^{b}	3404.58^{c}
Significance	*	*	*	NS	*	*	*	*	*	*	*	*

Table 4. Vegetative traits of five varieties of cucumber planted in May 2006

	Vine length (cm) WAP			No of branches/plant WAP			No of leaves/plant WAP			Leaf area cm^2/plant WAP		
Varieties	**4**	**6**	**8**	**4**	**6**	**8**	**4**	**6**	**8**	**4**	**6**	**8**
Market more 76	23.99^{c}	148.42^{b}	223.63^{ab}	1.23^{c}	7.32^{b}	10.44^{b}	7.75	33.61^{c}	39.44^{b}	906.32	3226.28^{c}	4022.52^{bc}
Ashley	28.86^{a}	168.45^{a}	244.02^{a}	1.43^{a}	13.23^{a}	14.76^{a}	8.22	40.72^{ab}	45.75^{ab}	993.24	4227.93^{c}	4879.70^{a}
Palmetto	28.78^{a}	155.45^{b}	232.71^{ab}	1.38^{ab}	12.36^{a}	13.14^{a}	8.14	42.77^{a}	47.20^{a}	963.61	3761.28^{b}	4349.76^{b}
Super marketer	26.21^{b}	150.26^{b}	$225.89b^{c}$	1.34^{b}	8.16^{b}	10.74^{b}	7.98	42.87^{a}	48.87^{a}	881.91	3264.43^{c}	4035.76^{bc}
Beith Alpha	26.13^{b}	144.99^{b}	217.43^{d}	1.23^{c}	8.76^{b}	10.23^{b}	7.76	35.73^{bc}	40.18^{b}	902.17	3015.56^{c}	3759.57^{d}
Significance	*	*	*	*	*	*	NS	*	*	NS	*	*

WAP = Weeks after planting

- Means followed by the same letter(s) within a treatment group are not significantly different at 5% level of significance using the Duncan Multiple Range Test (DMRT)
  NS Not significant
  * Significant at 5%

Table 5. Vegetative traits of five varieties of cucumber planted in June 2006.

Varieties	Vine length (cm) WAP			No of branches/plant WAP			No of leaves/plant WAP			Leaf area (cm^2)/plant WAP		
	4	6	8	4	6	8	4	6	8	4	6	8
Market more 76	20.26^{a}	158.17	233.93^{c}	0.89^{a}	6.22c	7.86^{d}	5.24^{b}	41.06^{b}	63.33^{b}	866.14^{b}	3536.84	3877.78
Ashley	24.01^{ab}	153.24	251.50^{ab}	1.03^{b}	9.24^{ab}	10.26^{b}	5.60^{b}	43.31^{b}	50.37^{b}	995.03^{a}	3757.49	3924.05
Palmetto	23.05^{b}	153.80	259.33^{a}	1.07^{b}	9.28^{b}	10.00b	4.47^{bc}	25.07^{a}	56.20^{a}	891.22^{a}	3746.75	3961.34
Super marketer	25.23^{a}	161.03	238.14^{bc}	1.25^{a}	10.14^{a}	10.80^{a}	6.14^{a}	40.75	47.78^{b}	891.54^{a}	3668.41	3603.86
Beith Alpha	21.15^{c}	150.82	238.10	0.89^{b}	8.64^{b}	9.22^{c}	5.27^{bc}	39.44^{b}	45.39	890.43^{b}	3635.63	3831.62
Significance	*	NS	*	*	*	*	*	*	*	*	NS	NS

* Significant at 5%
NS Not significant
Means followed by the same letter(s) within a treatment group are not significantly different at 5% level of significance using the Duncan Multiple Range Test (DMRT)

Table 6. Yield and yield components of five cucumber varieties planted in April 2006.

Varieties	No. of fruits/plant	Fruit length (cm)	Fruit girth (cm)	Wt of fruits/plant (kg)	Yield (kg/ha)
Market more 76	6.16^{c}	20.99	5.57^{b}	1.17	20,906.85
Ashley	9.53^{a}	19.04	5.93^{ab}	2.33	41098.03
Palmetto	8.59^{b}	20.40	5.96^{ab}	2.50	36565.93
Super marketer	6.57^{c}	20.51	5.50^{b}	1.59	28242.65
Beith Alpha	6.46^{c}	19.62	6.40^{a}	1.46	25917.35
Significance	*	NS	*	NS	NS

Means followed by the same letter(s) within a treatment group are not significantly different at 5% of significance using the (DMRT) Duncan Multiple Range Test
NS – Not Significant.

Table 7. Yield and yield components of five cucumber varieties planted in May 2006.

Varieties	No. of fruits/plant	Fruit length (cm)	Fruit girth (cm)	Wt of fruits/plant (kg)	Yield (kg/ha)
Market more 76	5.76[c]	14.64[b]	5.04	1.29	23431.54[b]
Ashley	8.50[a]	16.59[a]	5.65	2.37	29984.31[a]
Palmetto	7.48[a]	15.59[ab]	5.34	1.89	27940.31a
Super marketer	6.89[bc]	16.50[a]	5.46	1.41	24968.17[b]
Beith Alpha	5.60[c]	14.93[b]	4.90	1.38	24547.16[b]
Significance	*	*	NS	NS	*

*Means followed by the same letter(s) within a treatment group are not significantly different at 5% of significance using the Duncan Multiple Range Test (DMRT).
NS – Not Significant

Table 8. Yield and yield components of five cucumber varieties planted in June 2006.

Varieties	No. of fruits/plant	Fruit length (cm)	Fruit girth (cm)	Wt of fruits/plant (kg)	Yield (kg/ha)
Market more 76	3.84	15.76	4.40	1.00c	18213.70[c]
Ashley	6.66	15.74	4.77	1.68a	29892.08[a]
Palmetto	6.14	17.19	4.71	1.46b	26306.83[a]
Super marketer	5.19	15.74	4.78	1.39b	24783.46[b]
Beith Alpha	5.43	15.86	4.90	1.37b	24374.81[b]
Significance	NS	NS	NS	*	*

Table 9. Effect of planting dates on yield of five varieties of cucumber.

Varieties	April	May	June	Mean
Marke more 76	20906.85	23431.54	18213.54	20850.70
Ashley	41098.03	29984.31	29892.08	33658.14
Palmetto	36565.93	27940.31	26306.83	30371.02
Super marketer	28242.65	24968.17	24783.46	25998.09
Beith Alpha	25917.35	24547.61	24374.81	24946.59
Mean	30546.16	26174.39	24714.18	
LSD (P<0.05) Varieties 5739.23				
Planting dates 4445.59				
Varieties x Planting dates interaction Ns				

NS – Not Significant.

Conclusion

From the result of this study, it was observed that cucumber performs best in a forest savanna zone when planted early at the beginning of the wet season in April and Ashley variety is recommende for the area.

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