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COMPARATIVE STUDIES OF THE SUSCEPTIBILITY OF THE PODS OF SELECTED VARIETIES OF COWPEA TO *RIPTORTUS DENTIPES* F AND *ANOPLOCNEMIS CURVIPES* F (HETEROPTERA)

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ABSTRACT

The bugs, *Riptortus dentipes* and *Anoplocnemis curvipes* were reared on two cowpea varieties namely Sokoto white and TVx 3236. The bugs were observed for their growth, oviposition, progeny (neanide) development, and damage on the pods on the field. These bugs showed high levels of oviposition and neanide development on Sokoto white variety than on TVx 3236 variety. Five *R. dentipes* female bugs reared on Sokoto white pods laid 386±2.10 eggs within 4 weeks while 290±1.01 eggs were laid within the same period on TVx 3236 pods. Five *A. curvipes* female bugs reared on Sokoto white and TVx 3236 pods laid 491±2.25 eggs and 394±2.00 eggs respectively within the same period of 4 weeks. Faster growth of neanide and adults were observed on Sokoto white pods than on TVx 3236 pods for both bugs. It took *R. dentipes* bugs reared on Sokoto white pods shorter days (25±0.3 days) than on TVx 3236 (29±1.1) to develop from egg stage to adult stage. *A. curvipes* took 31.5±0.5 days and 36±1.5 days to reach adult stage on Sokoto white pods and TVx 3236 pods respectively. Both adult and neanides preferred younger pods which they sucked thereby causing the pods to abort and dry prematurely.

Key words: *Riptortus dentipes, Anoplocnemis curvipes,* oviposition, neanide and Sokoto white variety

INTRODUCTION

Cowpea, *Vigna unguiculata* (L) Walp, is an indigenous crop of Africa where it is eaten as processed dry grain, green pod and tender green leaves (Lawani, 1989). Cowpea grain has high protein content (about 24%) and is rich in other essential nutrients such as calcium, iron, thiamine and riboflavin (Lawani, 1989). It is an important source of human dietary protein and livestock feeds in the tropics where consumption rate of animal protein and per capital income are very low (Okigbo 1978). Many improved and local cultivars of cowpea cultivated in Nigeria include TVx3236, KN-1, Ife Bimpe, Ife Brown, SUVITA-Z, TN88-63 while the local varieties include Sokoto white, Sokoto brown, Kano white, Kano brown, Kudi and Kwara. Several insect pests and diseases which co-evolved with cowpea cause major setback in obtaining full yield of the crop (Singh 1988b). Lawani (1989) reported that many diseases and insects that, attack cowpea plants and stored grains plague the cowpea plants and damage the seeds. Singh (1988a) reported that major field pests of cowpea in Africa include aphids, flower thrips, pod borers and pod sucking bugs. However, the post-flowering pests of cowpea cause the greatest yield losses ranging between 50%-85% on the field (Singh 1988b). The damage of cowpea by pests and diseases have caused severe quantitative and qualitative losses.

Many studies have been conducted on the effectiveness of synthetic chemicals, plant parts, oils, and the use of biological predators as potent protectants of stored legumes (Golob 1991,1992, Daniel and Smith 1994, Ogunwolu and Odunlami 1996, Alebeek 1996, Kitch et.al. 1997, Zetter et.al. 1997, Omotoso 2004, 2005). The use of synthetic chemicals and biological predators are very costly and are beyond the reach of peasant farmers (Giga and Biscoe 1989, Alebeek 1996). The development and the use of resistant legume varieties offer a simple, cheap and attractive approach to the reduction of pests damage (Ofuya et.al. 2001). The potential of using host plant resistance in cowpea for the control of pests have received considerable attention (Dobie, 1986; Singh et.al, 1990; Ofuya, 1995a, 1995b). Although crop varieties certainly differ in their susceptibility to different pests (Lale and Kolo, 1998), it is very difficult to find a single perfect variety which would meet all the farmers requirements, such as high yield, resistance to field pests and diseases, good processing and cooking qualities, palatability as well as durability in storage (Compton et. al, 1993).

R. dentipes and *A.curvipes* are hemipteran pod-sucking bugs which cause abortion and premature drying of cowpea pods on the field. These bugs are the most destructive pests of developing pods of cowpea on the field (Omotoso,

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1997). It is against the dearth of information on the effectiveness of the use of resistant cowpea varieties against pod sucking bugs that this study was conducted to elucidate the comparative susceptibility of the pods of selected varieties of cowpea to *R.. dentipes* and *A. curvipes.*

MATERIALS AND METHODS

INSECT CULTURE

R. dentipes and *A. curvipes* used to establish the cultures were obtained from the cowpea farm cultivated on the campus of the University of Ado-Ekiti, Ekiti State, Nigeria. The bugs were collected with insect nets. The bugs were reared on uninfested fresh pods of Sokoto white local variety and TVx 3236 variety in plastic boxes measuring 13.5x8.5x8cm in the laboratory. The boxes were covered with nylon mesh and held tightly with rubber bands. The insects were reared under flunctuating ambient temperatures (28±2°C) and relative humidities (75±5%) in the laboratory. New generations of the bugs obtained from the eggs laid by the females were used as replacement for the dead ones. Also, devoured pods and those on which bugs laid their eggs were continuously replaced with fresh uninfested ones.

FEEDING AND OVIPOSITION ASSAYS FOR *R. dentipes*

Ten fresh pods of Sokoto white pods were put in boxes (12x10x10cm). Also, in another set of boxes were 10 fresh pods of TVx 3236. Five pairs of newly emerged adults of *R. dentipes* (i.e. 5 males and 5 females, aged 0-1h) were put in each of the boxes and covered with nylon mesh. Devoured pods and those on which eggs were laid were removed to new boxes while they were replaced with fresh pods. The bugs were fed till when they started laying eggs. Daily observations were made on their feeding and oviposition behaviours and the experiment was terminated after 4 weeks of oviposition. Each of the experiments was in triplicates.

FEEDING AND OVIPOSITION ASSAYS FOR *A. curvipes*

Ten fresh pods of Sokoto white pods were put in boxes. Also, 10 fresh pods of TVx 3236 were put in another set of the boxes. Five pairs of newly emerged adults of *A. curvipes* (i.e. 5 males and 5 females, aged 0-1h) were put in each of the boxes and covered with nylon mesh. Devoured pods and those with laid eggs were removed to new boxes while they were replaced with fresh pods. The bugs were fed till when they started laying eggs. Daily observations were made on their feeding and oviposition behaviours and the experiment was terminated after 4 weeks of oviposition. Each of the experiments was in triplicates.

PROGENY EMERGENCE (HATCHABILITY) ASSAY FOR *R. dentipes*

Ten fresh pods of Sokoto white local pods and TVx 3236 were put in separate boxes. Five pairs of newly emerged adults of *R. dentipes* (i.e. 5 males and 5 females, aged 0-1h) were put in each of the boxes. Devoured pods and the eggs laid were removed to new boxes. Eggs laid within 2 weeks were allowed to hatch and the insects that subsequently emerged from the eggs were counted to estimate the F1 progeny production. Each of the experiments was in triplicates.

PROGENY EMERGENCE (HATCHABILITY) ASSAY FOR *A. curvipes*

Ten fresh pods of Sokoto white local pods were put in boxes. Also, 10 fresh pods of TVx 3236 pods were put in another set of boxes. Five pairs of newly emerged adults of *A. curvipes* (i.e. 5 males and 5 females, aged 0-1h) were put in each of the boxes. Devoured pods and the eggs laid were removed to new boxes. Eggs laid within 2 weeks were allowed to hatch and the insects that subsequently emerged from the eggs were counted to estimate the F1 progeny production. The progenies were reared on fresh pods daily for 4 weeks when the male to female sex ratio was determined. Each of the experiments was in triplicates.

NEANIDE GROWTH AND DEVELOPMENT ASSAYS FOR *R. dentipes*

Ten eggs laid on fresh pods of Sokoto white pods and TVx 3236 pods by *R. dentipes* were carefully removed and put in new boxes separately. The eggs were allowed to hatch in the laboratory. Immediately after hatching, 10 fresh pods of Sokoto white local pods and TVx 3236 pods were put inside the respective boxes and covered with nylon mesh. Devoured pods were replaced with fresh ones daily and the neanide development were monitored for 8 weeks. Each of the experiments was in triplicates. Data were collected on the development of the neanides.

NEANIDE GROWTH AND DEVELOPMENT ASSAYS FOR *A. curvipes*

Ten among the eggs laid by *A. curvipes* on Fresh pods of Sokoto white local pods and TVx 3236 pods were carefully removed and put inside new boxes separately. The eggs were allowed to hatch in the laboratory. Immediately after hatching, 10 fresh pods of Sokoto white local pods and TVx

3236 pods were put inside the respective boxes and covered with nylon mesh. Devoured pods were replaced with fresh ones daily and the neanide development were monitored for 8 weeks. Each of the experiments was in triplicates. Data were collected on the development of the neanides.

RESULTS AND DISCUSSION

The result of the oviposition of *R. dentipes* on Sokoto white variety and TVx 3236 variety are shown in Fig. 1. The result shows that the bugs laid more eggs on Sokoto variety than on TVx 3236 variety, indicating more attacks on the local variety. Five females *R. dentipes* bugs laid 386±2.10 eggs on Sokoto white pods while 303±1.01 eggs were laid on TVx 3236 pods respectively within 4 weeks. The bugs laid the highest number of their eggs between days 13 and 16. *R. dentipes* sucked both old and young pods, thereby causing the pods to shrivel and dry prematurely. Both mated and unmated females of *R. dentipes* laid eggs but the eggs laid by unmated females been sterile, failed to develop beyond the egg stage.

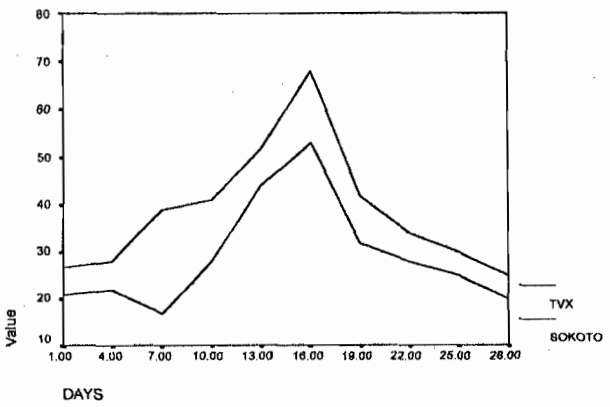

FIG. 1: OVIPOSITION OF FEMALE *R. dentipes* ON SOKOTO AND TVx 3236 VARIETIES OF COWPEA IN 4 WEEKS

The number of eggs deposited by the females decrease as the females advanced in age. The bugs have ovipositional preferences for Sokoto variety which they devoured seriously. Nwanze et.al.(1975) has earlier reported this same observation in *Callosobruchus maculatus,* that they have ovipositional preferences for some varieties of cowpea.

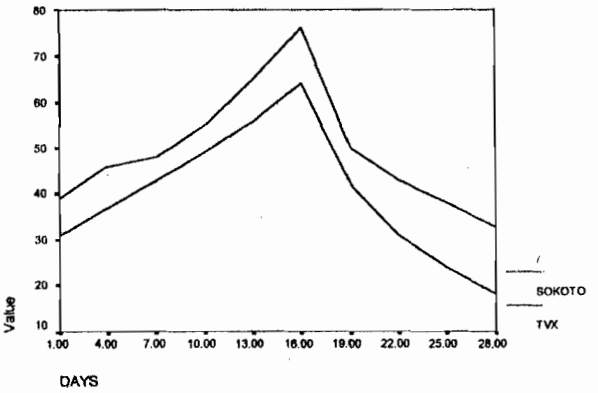

FIG. 2: OVIPOSITION OF FEMALE *A. curvipes* ON SOKOTO AND TVx 3236 VARIETIES OF COWPEA IN 4 WEEKS

Fig. 2 shows the result of oviposition of *A. curvipes* for 4 weeks on the cowpea varieties. Only the mated females of *A. curvipes* laid eggs. *A.curvipes* however preferred older pods, which they sucked thus, causing the seeds inside them to shrivel. Adeniji et al (1991b) reported that sucking causes the shrivelling and premature abortion of cowpea pods on the field. Five females *A. curvipes* bugs laid 491±2.25 eggs on Sokoto white local variety while 394±2.00 eggs were laid on TVx 3236 variety. The bulk of their eggs were deposited within the 13th and the 16th days.

Fig. 3. shows the hatchability of the eggs laid by female *R. dentipes* within 2 weeks. A total of 171±1.10 eggs laid on TVx 3236 pods and 207±2.21 eggs laid on Sokoto white pods by *R. dentipes* hatched out. The eggs in the same batch hatched out within 2 minutes and the neanides, which emerged, aggregated at first for 4-5 days before dispersing.

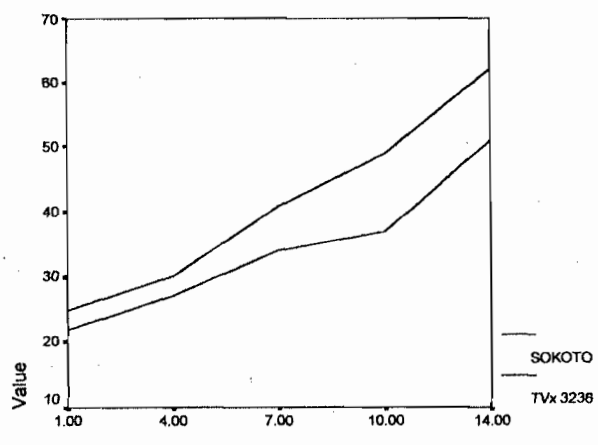

FIG. 3: HATCHABILITY OF *R. dentipes* ON SOKOTO AND TVx 3236 VARIETIES OF COWPEA IN TWO WEEKS

Fig. 4 shows the hatchability of the eggs laid by female *A. curvipes* within 2 weeks. The sum of 207±1.05 eggs laid by the bugs on Sokoto white local pods and another 202±2.00 eggs laid by another 10 bugs on TVx 3236 within 2 weeks hatched out.

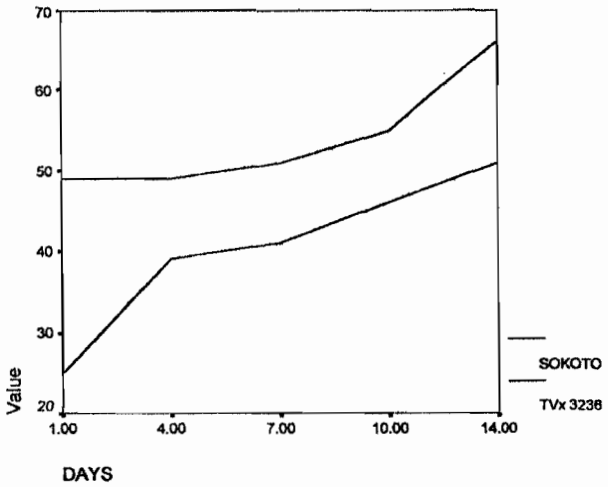

Fig. 4: HATCHABILITY OF *A. curvipes* ON SOKOTO AND TVx 3236 VARIETIES OF COWPEA IN TWO WEEKS

The eggs in the same batch hatched out within 2 minutes and the neanides, which emerged, aggregated at first for 4-5 days before dispersing to search for food.

Table 1. shows the egg and neanide periods of *R. dentipes* on Sokoto and TVx 3236 varieties of cowpea. The table shows that egg incubation was 7±0.5 days on Sokoto variety while it was 8±0.5 days on TVx 3236. Egg incubation and adult emergence took longer periods in TVx 3236 (8±0.5 days and 29±1.1 days respectively) than in Sokoto white pods (7±0.1 days and 25±0.3 days respectively. This may have been the effect of the resistance of the breed to bugs infestation. The male to female sex ratio in *R. dentipes* reared on TVx 3236 and Sokoto white pods was 1.01: 1 and 1: 1.01 respectively.

TABLE 1 EGG AND NEANIDE PERIODS OF *R. dentipes* REARED ON TWO VARIETIES OF COWPEA

Cowpea	Egg incub.	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
Sokoto	7	2	4	3	3	6
TVx 3236	8	3	4	3	3	8

Table 2 shows the egg and neanide periods of *A. curvipes* reared on Sokoto and TVx 3236 varieties of cowpea. Egg incubation took 6 and 7.5±1.5 days on Sokoto and TVx 3236 varieties respectively. There were 5 neanide stages before the adults emerged.

TABLE 2 EGG AND NEANIDE PERIODS OF *A. curvipes* REARED ON TWO VARIETIES OF COWPEA

Cowpea	Egg Incub.	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
Sokoto	6	2.5	4.5	4	5	9.5
TVx 3236	7.5	3.5	5.5	4	5.5	10

Eggs incubation and adults emergence took longer periods in TVx 3236 (i.e. 7.5±0.2 and 36±1.5 respectively) than in Sokoto white local variety (i.e. 6.05±0.1 and 31.5 ±0.5) respectively. The male to female sex ratio in the *A. curvipes* reared on TVx 3236 was 1:1.01 while it was 1.02:1 on Sokoto white local pods

These bugs performed very well in the laboratory because their developments were favoured by the laboratory conditions. The tropical environmental conditions have been reported to favour optimal development of pests hence pest problems are so severe in the region (De Lima 1987). In addition, the temperature of the laboratory, which ranged between 20°C- 45°C have been found to speed up biological activities of storage pests in the tropics (Ofuya 2001). One possible explanation for the fewer number of eggs output by the bugs reared on TVx 3236 is the effect of the resistance of the variety to the bugs. The resistance of some selected cowpea seeds to *C. maculatus* had been confirmed by several authors (Ofuya and Credland 1995a, Adeduntan et al 1998). An appreciable suppression of the growth and development of *R. dentipes* and *A. curvipes* were achieved with TVx 3236 variety, however, further trials are recommended to determine the effects of resistant varieties of other crops on the growth, oviposition and development of their insect pests.

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REFERENCES

Adeduntan, S.A and Ofuya, T.T.(1998). Evaluation of Seeds of selected varieties of cowpea, *vigna unguiculata(L)* Walp for susceptibility to *Callosobruchus maculatus*(Fabricius) (Coleoptera: Bruchidae*). Applied Tropical Agriculture* 3: 45-51

Adeniji, M.O, Nwabeke, P.N., Iheukwumere, F and Udeogalanya, A.C.C.(1991b). Agriculture Science Book 1. 2nd Edit. Published by Evans Brothers (Nigeria Publishers) Ltd. Pp.24-53.

Alebeek, F.A.N van. (1996). Foraging behaviour of the egg parasitoid Uscana *lariophaga* ,Ph.D.Thesis, Wageningin Agricultural University,The Netherlands.176pp.

Compton, J.A.F., Tyler, P.S., Golob, P., Boxall, R.A and Haines, C.P. (1993). Reduction losses in small farm grain storage in the tropics. *Tropical Science* 33: 283-318.

Daniel, S.H and Smith, R.H. (1994). Functional anatomy of the pore in *Callosobruchus maculatus*:a trade-off between gas exchange and protective functions.*Physiological Entomology* 19: 30-38.

De-Lima, C.P.F. (1987). Insect pests and postharvest problems in the tropics.*Insect Science and its Application* 8: 673-676.

Dobie, P.V. (1986). Potential uses of host plant resistance.In: E.Donabaye and S.Navarrot (eds),*Proceedings of 4th International working Conference on stored Products Protection.*Tel. Aviv, Israel pp 2-13.

Giga, D.P and Biscoe, J. (1989). Treating maize grain for storage with registered protectants in Zimbabwe: Technical, practical and economic considerations. *Zimbabwe News* 23: 101-103.

Golob, P. (1991). An evaluation of the campaign to control the larger grain borer,*Prostephanus truncatus*(Horn) in Western Tanzania. *FAO Plant Protection Bulletin* 39: 65-71.

Golob, P. (1992). The larger grain borer -its impact on maize marketing and storage pest control. *Tropical Agriculture* Association *Newsletter* 12: 10-11.

Hodges, R.K., Mik, J. and Denton, H. (1985). Infestation of dried cassava (*Manihot esculenta* Crantz) by *Prostephanus truncatus. Jour.of Stored Products Research* 21: 73-77.

Kitch, L.W., Bottenberg, H and Wolfson, J.L. (1997). Indigenous knowledge and cowpea pest management in sub-Saharan Africa. pp. 292-301:In B.B. Singh,D.R. Moham-Raj,K.E.Dashiell and L.E.N.Jackai(eds),Advances in Cowpea Research. IITA,Ibadan, Nigeria & JIRCAS,Tsukuba,Japan.

Lale, N.E.S and Kolo, A.A. (1998). Susceptibility of eight genetically improved local cultivars of cowpea to *Callosobruchus maculatus* F(Coleoptera:Bruchidae) *International Jour. of Pest Management* 44: 25-27.

Lawani, S.M. (1989). The cowpea Biotechnology and Natural Pest Control. IITA Research Briefs. 9: 1-6.

Nwanze, K.F., Horber, E and Pitts, C.W. (1975). Evidence for ovipositional preference of *Callosobruchus maculatus* for cowpea varieties. *Environmental Entomology.* 4: 409-412.

Ofuya, T.I and Credland, P.F. (1995a). Differences in the susceptibility of seeds of selected varieties of cowpea to *Bruchidius atrolineatus* (Cleoptera: Bruchidae). *Bulletin of Entomological Research* 85: 259-265.

Ofuya, T.I and Credland, P.F. (1995b). Response of three populations of the seed beetle,*Callosobruchus maculatus* (L) Walpers. *Jour. of Stored Products Research* 31: 17-27.

Ofuya, T.I. (2001). Biology, ecology and control of insect pests of stored food legumes in Nigeria.In: T.I.Ofuya,and N.E.S.Lale(Eds),Pests of stored cereals and pulses in Nigeria. 1st edt. Published by Dave Collins Publications,Akure, Nigeria. Pp1-58.

Ogunwolu, E.O and Odunlami, A.T. (1996). Suppression of seed bruchid (*Callosobruchus maculatus* F) development and damage on cowpea(*Vigna unguiculata* (L) Walp.) with *Zanthoxylum zanthoxyloides* (Lam.) Waterm (Rutaceae) root bark powder when compared to neem seed powder and pirimiphos-methl. *Crop Protection* 15: 603-607.

Okigbo, B.N. (1978). Grain Legumes in the agriculture of the tropics. In S.R. Singh, H.F. Van Emden and T.A. Taylor (eds), Pests of grain Legumes: Ecology and control. Academic Press, London pp. 454.

Omotoso, O.T. (1997). The life histories of two pod sucking bugs of cowpea *Anoplocnemis curvipes* F (Heteroptera : Coreidae) and *Riptortus dentipes* F. (Heteroptera: Alydidae). M.Sc , Thesis, University of Ado-Ekiti.

Omotoso, O.T. (2004). Evaluation of some plant powders as toxicants and antifeedants in the control of maize damage by the maize weevil, *Sitophilus zeamais* Motschulsky in storage. *Ultra Science* 16(2): 221-230.

Omotoso, O.T. (2005). Determination of susceptibility index of ethanoic extracts of some plants to maize weevils, *Sitophilus zeamais way* Motschulsky. *Journal of Science and Technology Research* 4(1): 16-20.

Singh, S.R. (1988a). Cowpea varieties for different Agroecological zones and cropping systems. *IITA Research Highlights* 8(88): 76-81.

Simgh, S.R. (1988b). Host plant resistance of cowpeas to post-flowering insect pests. *IITA Research Highlights* 8: 70-75.

Singh, B.B and Singh, R.B. (1990). Breeding for bruchid resistance in cowpea.In: K.Fujii, A.M.R.Gatehouse, C.D. Johnson, R. Mitchell and T. Yoshida (Eds.) Bruchids and Legumes: Economic,Ecology and Co-evolution, Kluwer Academic Publishers, Dordrecht, pp. 219-228.

Zetter, J.L., Leesch, J.G., Gill, R.F and Mackey, B.E. (1997). Toxicity of carbonyl sulfide to stored product insects. *Jour. of Economic Entomology* 90: 832-836.

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BACTERIOLOGICAL ANALYSIS OF BOREHOLE WATER IN ULI, NIGERIA

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ABSTRACT

Water samples from four different boreholes within Uli, Anambra State, Nigeria, were collected for five consecutive weeks for bacteriological analysis to assess the potability.The range of the means was from $1.5x10^2$ to $5.9x10^4$ cfu ml^{-1} for total aerobic bacterial load, 9 to 136 MPN per 100ml for total coliforms and 4 to 74 MPN per 100ml for faecal coliforms. The highest counts were consistently found in the sample from Cagramento Lodge, where the borehole was located in an unsanitary environment, near a pit laterine. *Escherichia coli, Klebsiella sp., Proteus sp., Enterobacter sp., Pseudomonas sp.,* and *Staphylococcus aureus* were isolated from the samples. The findings show that the water from all the boreholes did not meet the World Health Organization Standards for Drinking Water and should be treated or boiled and filtered before drinking.

Key words: Faecal coliforms, borehole water, Uli, Nigeria

INTRODUCTION

In developing countries including Nigeria, where the majority of people live in rural areas, rivers, streams, wells and more recently boreholes, serve as the main sources of water for drinking and domestic use. The underground water supplies are usually considered safe provided they are properly located, constructed and operated according to the World Health Organization Guidelines for Drinking Water (WHO, 1971).

Main origins of pollution of wells and boreholes are industrial, domestic and agricultural and pollution can be continuous or accidental. Industrial pollution may involve seepage of used water containing chemicals such as metals and radioactive compounds, or contaminated water from damaged pipelines infiltrating into the borehole. Domestic pollution may involve seepage from broken septic tanks, pit latrines, cesspools and privies. Agricultural pollution is from irrigation water and run off water after rains, carrying fertilizers, pesticides, herbicides and faecal matter. Environmental pollution is mainly from sea water intrusion into coastal aquifer. The WHO recommends that boreholes should be located at least 30m away from latrines and 17m from septic tanks (Chukwurah, 2001; Wagner and Lanoix, 1969).

Microorganisms of concern in contaminated water include the following bacterial agents of diarrhea and gastroenteritis namely *Salmonella sp., Shigella sp., Escherichia coli and Vibrio cholerae (*Birmingham *et al.,* 1997). Protozooal agents of diarrhea include *Entamoeba histolytica, Giardia lamblia, Balantidium coli* (Jawetz *et al.* 1991) *and Cryptococcus pervum* (Kelly *et al.* 1997). Enteroviruses causing various clinical ailments, not necessarily diarrhea, but are transmitted by water include Poliovirus, Rotavirus, Hepatitis A virus (Hejkal *et al.* 1982) and Hepatitis E virus (Benjelloun *et al.* 1997).

Presence of faecal coliforms or *Escherichia coli* is used as an indicator for the presence of any of these water borne pathogens (Chukwurah, 2001; Okafor, 1985; Okpokwasili and Akujobi, 1996). WHO recommends that no faecal coliform be present in 100ml of drinking water. Good quality water is odourless, colourless, tasteless and free of faecal contamination and chemicals in harmful amounts. This study was therefore carried out to determine the bacteriological quality of water from boreholes located in the vicinity of the Anambra State University of Science and Technology, Uli. Students and other members of the community pay for the water and it was important to find out if the water was safe for drinking and domestic use and to recommend treatment if necessary or suggest measures to be taken to eliminate the source of pollution.

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MATERIALS AND METHODS

SAMPLING LOCATIONS

Water samples were collected from boreholes used as sources of domestic water by students and other members of the community in Uli town in Ihiala Local Government Area of Anambra State, Nigeria. The samples were collected at weekly intervals for 5 weeks from the following students' hostels: (A) Victory Lodge, (B) Doggy Hostel, (C) Cagramento Lodge and (D) Global Hostel.

COLLECTION OF SAMPLES

Cotton wool soaked in 70% (v/v) ethanol was used to sterilize the nozzle of the borehole from which samples were collected. The tap was allowed to run for two minutes before sterile 250ml screw capped glass bottles were carefully uncapped and filled with the water and recapped. Water samples were transported to the laboratory in a cooler with ice for bacteriological analysis within two hours of collection.

TOTAL HETEROTROPHIC BACTERIAL COUNT

The spread plate method was used. Water samples were serially diluted using sterile 1ml pipettes and 9ml sterile physiological saline as diluent. Aliquots of 0.1ml of undiluted water sample and water at dilutions of 10^{-1} and 10^{-2} were plated on Nutrient agar (Oxoid) plates in duplicates. The plates were incubated at 37^0C for 24h, before enumeration.

TOTAL COLIFORM AND FAECAL COLIFORM COUNT

PRESUMPTIVE TEST

Coliform count was obtained using the three tube assay of the Most Probable Number (MPN) technique (Speck, 1976). Presumptive coliform test was performed using MacConkey broth (Oxoid). The first set of three tubes had sterile 10ml double strength broth and the second and third sets had 10ml single strength broth. All the tubes contained Durham tubes before sterilization. The three sets of tubes received 10ml, 1ml and 0.1ml quantities of water samples using sterile pipettes. The tubes were incubated at 37^0C for 24-48h for estimation of total coliforms and at 44.5^0C for faecal coliforms for 24-48h and examined for acid and gas production. Acid production was determined by color change of the broth from reddish purple to yellow and gas production was checked for by entrapment of gas in the Durham tube.

The MPN was then estimated from the MPN table for three tube test.

CONFIRMED TEST

Confirmed test was carried out by transferring a loopful of culture from a positive tube from the presumptive test into a tube of Brilliant Green Lactose Bile (BGLB) broth (Oxoid) with Durham tubes. The tubes were incubated at 37^0C for 24-48h for total coliforms and 44.5^0C for faecal coliforms and observed for gas production.

COMPLETED TEST

Completed test was carried out by streaking a loopful of broth from a positive tube onto Eosine Methylene Blue (EMB) agar plate for pure colonies. The plates were incubated at 37^0C for 24-48h.Colonies developing on EMB agar, were further identified as coliforms or faecal coliforms (*Escherichia coli*) using cultural characteristics, morphology and biochemical tests. For faecal coliforms, colonies with green metallic sheen were Gram stained and the IMViC test was carried out on Nutrient agar stock cultures and used to identify the colony as *E. coli*. The MPN per 100ml water was calculated using the completed test.

IDENTIFICATION OF BACTERIAL ISOLATES

Stock cultures of the isolates with different cultural characteristics were made on nutrient agar slants. Gram staining was used to check for morphology and biochemical tests were performed to aid in identification. Various tests performed and used in probable identification of isolates included the oxidase test, motility test, catalase test, urease test, coagulase test, indole test, methyl red test, Voges-Proskauer test and citrate utilization test (Treagan and Pulliam, 1982).

RESULTS

Table 1 shows the range and mean values of total bacterial counts, total coliform counts and faecal coliform counts of water samples collected at weekly intervals over a period of 5 weeks. Total heterotrophic bacterial counts of the four boreholes (A, B, C and D) were relatively low except for sample C, the Cagramento Lodge borehole. The counts for sample A ranged from 4.0 x 10 -2.5 x 10^2 cfu ml^{-1} with a mean value of 1.4 x 10^2 cfu ml^{-1}, sample B ranged from 2.0 x 10 – 1.6 x 10^3 cfu ml^{-1} with a mean value of 4.8 x 10^2 cfuml^{-1}, sample C which had the highest values ranged from 2.5 x 10^3 – 1.5 x 10^5cfu ml^{-1} with a mean value of 5.9 x 10^4 cfu ml^{-1} and sample D ranged from 5.0 x 10 – 2.5 x 10^2 cfu $ml^{-1}$with a mean value of 1.5 x 10^2 cfu ml^{-1}.

TABLE 1 THE RANGE AND MEAN VALUES OF TOTAL BACTERIAL COUNTS, TOTAL COLIFORM COUNTS, AND FAECAL COLIFORM COUNTS

Water sources	Total heterotrophic bacteria count (cfu ml^{-1})	Total coliform counts (MPN/100ml)			Faecal coliform count (MPN/100ml)	
	Range	Mean	Range	Mean	Range	Mean
A	$4.0 \times 10 - 2.5 \times 10^2$	14×10^2	9-11	10	3-7	5
B	$2.0 \times 10 - 1.6 \times 10^3$	4.8×10^2	7 –11	9	3 –7	4
C	$2.3 \times 10^3 - 1.5 \times 10^5$	5.9×10^4	64-240	136	64-93	74
D	$5.0 \times 10 - 2.5 \times 10^2$	1.5×10^2	11	11	7	7

Total coliform counts for the samples were also highest sample C, with a mean count of 136 MPN per 100ml while samples A, B and D had mean counts of 10, 9 and 11 MPN per 100ml respectively.

Faecal coliform count was highest for sample C, with a mean value of 74 MPN per 100ml. Samples A, B and D had values of 5, 4 and 7 MPN per 100ml respectively.

Based on the cultural characteristics, morphology and the results of biochemical tests, six isolates were identified as *Proteus sp., Escherichia coli, Pseudomonas sp., Enterobacter sp., Staphylococcus sp. and Klebsiella sp.* as shown in Table 2.

TABLE 2 CHARACTERIZATIONS AND POSSIBLE IDENTIFICATION OF ISOLATES FROM BOREHOLE WATER

Isolates	Morphology	Gram Stain	Urease	Methyl Red	Indole	VP	Citrate	Catalase	Oxidase	Coagulase	Motility	Glucose	Maltose	Probable Identification
1	Rods	-	+	+	+	-	-	+	-	-	+	A	A	*Proteus sp*
2	Rods	-	-	+	+	-	-	+	-	-	+	A/G	-	*Escherichia coli*
3	Rods	-	-	-	-	-	-	+	+	-	+	A	-	*Pseudomonas sp*
4	Rods	-	-	-	-	+	+	+	-	-	+	A/G	A/G	*Enterobacter sp*
5	Cocci	+	-	+	-	-	-	+	-	+	-	A	A	*Staphyiococcus sp*
6	Rods	-	+	-	-	+	+	+	-	-	-	A/G	A/G	*Klesiella sp*

DISCUSSION

Water suitable for human consumption (potable water) should be free from disease producing organisms or large numbers of non-pathogenic organisms.

The borehole water from three locations (Victory Lodge, Doggy Lodge, Global Allen) had considerably lower heterotrophic bacterial counts and total coliform counts and could be concluded to be of better quality for domestic use than the Cagramento Lodge water which had much higher counts of both bacteriological parameters.

Regarding the faecal coliform counts, even though the Cagramento water had much higher mean value of 74 MPN per 100ml compared to counts of 4 to 7 MPN per 100ml for the other 3 boreholes, it can be concluded that water from all the boreholes are not fit for drinking without processing (WHO, 1971; WHO, 1986; USEPA, 2001). WHO and United States Environmental Protection Agency Standard for faecal coliform in drinking water is zero faecal coliform per 100ml. Therefore water from all the boreholes should be boiled and filtered for clarity before drinking.

The observations in this study support the fact that high heterotrophic counts in water reflect high coliform counts and the presence of faecal coliforms. The presence of high faecal coliform count in sample C could be attributed to the proximity of the borehole to a pit latrine located near the borehole at a distance less than the 30m recommended by WHO and the general unhygienic environment surrounding the borehole. It could be that the pipes used for water distribution were rusty thus allowing seepage of microbial contaminants into the borehole. In addition, the bacterial isolates from the water

belong to genera of potential pathogenic bacteria, hence the recommendation that water from all the boreholes need to be boiled before use.

There is need to increase awareness of the community towards the dangers associated with the use of contaminated water; the danger in constructing pit latrines and septic tanks near a water source and *vice versa;* the use of rust-free polyvinyl chloride (PVC) pipes for water distribution and treatment of water by boiling and filtering before use for drinking and cooking.

REFERENCES

Benjelloun, S., B. Bahbouhi, N. Bouchrat, L. Chericaoni, N. Had, J. Mahjour, and A. Bensumane (1997) Seroepidemiological study of an acute Hepatitis E outbreak in Morocco. *Research Virology* **148**: 279-283.

Birmingham, M.E., L. A. Lea, N. Ndayiminje, S. Nkurikiye, B.S. Hersh, J.G.Wells and M.S. Ijeming (1997) Epidemic cholera in Burundi, Patterns of transmission in the Gadat Rift Valley Lake Region. *Lancet* **349**: 981-983.

Chukwurah, E.I. (2001) *Aquatic Microbiology*. Otoba Press Limited, Onitsha, Nigeria.

Hejkal, T.W., B. Keswick, R.L. Labelle, C.P. Gerba, V. Sanchez, G. Dreesman, B. Hafkin and J.L. Melnick (1982) Viruses in a community water supply associated with an outbreak of gastroenteritis and infectious Hepatitis. *Journal of the American Water Works Association* **74**: 317-321.

Jawetz, E., J.L. Melnick and E.A. Adelberg (1991) *Medical Microbiology* (19th ed.) Appleton and Lange, Norwalk. Connecticut.

Kelly, P.B., K. S. Ndubani, P. Nchito, N.A. Luo, R.A. Feldman and M.J. Farthing (1997) Cryptosporidiosis in adults in Lusaka, Zambia and its relationship to oocyst contamination of drinking water. *Journal of Infectious Disease* **176**: 1120-1125.

Okafor, N. (1985) *Aquatic and Water Microbiology*. Fourth DimensionPublishers, Enugu, Nigeria.

Okpokwasili, G.C. and T.C. Akujobi (1996) Bacteriological indicators of tropical water quality. *Environ. Toxicol. Water Quality* **11**: 77-82.

Speck, M.L. (1976) *Compendium of Methods for the Microbiological Examination of Foods*. American Public Health Association, Washington, DC.

Treagan, L. and L. Pulliam (1982) *Medical Microbiology Laboratory Procedures*. W.B. Saunders Co. Philadelphia.

USEPA (2001) Current Drinking Water Standards. United States Environmental Protection Agency, Washington, DC.

Wagner, E.G. and J.N. Lanoix (1969) Water Supply for World Health Organization General Monograph Series No. 42.

WHO (1971) Guideline for Drinking Water Quality. World Health Organization. Geneva.

WHO (1986) International Standards for Drinking Water Quality. World Health Organization. Geneva **1**:99-120.