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Effect of varying moisture content of kolanut on kolanut weevil: *Balanogastriskolae* (Desbr) (Coleoptera: Curculionidae)

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Abstract
This research work focused on the evaluation of feeding rate of kolanut weevil Balanogastris kolae on different moisture content levels of two Cola species (Cola. accuminata and C. nitida) during storage after infestation. Through a weighing method and the survival rate with a view of finding alternative means of storage to reduce economic loss encountered by most farmers. During the experiment, uninfested and infested (source of infestation) kolanuts were sampled and assessed using these parameters percentage moisture content and percentage weight loss from the two Cola species. Another set up consisting C. acuminate and C. nitida using male and female B. kolae in the ratio of 2 to 3 was introduced into it to study the biology of B. kolae on each species of kolnut with respect to their moisture content. C. acuminate was observed to have a higher percentage moisture content when compare to the C. nitida but not significantly different ($p>0.05$).It was recorded that, the weight of kolanut with higher moisture content reduced significantly ($p<0.05$) with time after infestation with B. kolae during storage. There was significant different between the total mean weight loss of C. acuminate **(**216.77 ± 70.14) and C. nitida (341.47±102.86) after infestation at $p=0.022$. Hundred percent (100%) level of weevil infestation and nut damage was recorded in kolanut with higher moisture content compared to about 40% level infestation and nut damage observed in kolanuts with reduced moisture content for the two species. Progeny emergence was observed first in C. acuminate within the first week of their life cycle study while C. nitida had higher percentage of total emergence. These research findings confirm that high moisture contents on both Cola species encourages weevil attack. There is therefore, an urgent need to use reduced moisture content method as an alternative method of storage rather than subjecting kolanuts to chemical treatments for the control of kolanut weevils.

Keywords: *balanogastris kolae, kolanut,* moisture content and infestation

1. Introduction

Kolanut is indigenous to the tropical rain forest of West Africa, West Indies, Brazil and Java (Rusell, 1955) and belongs to the plant family *Steruliacea,* (Ogutuga, 1975; Egbe and Oladokun, 1987 [14] and Odebode, 1996). About 40 *Cola species* have been described in West Africa. However, *Cola acuminate and C. nitida* are two *Cola species* of real importance in Nigeria (Quarrco, 1973; Daramola, 1978 [6]; Agatha *et al*., 1978 and Arogba, 1999). Outside West Africa, cultivation and trade in kolanut have been established in countries like the Caribbean Islands Mauritius, Sri-Lanka and Malaysia (Oladokun, 1982) [14]. In the forest areas of West Africa, kola is perhaps second only to palm oil in importance in the list of indigenous cash crops. Unfortunately, kolanut still remains the only indigenous African cash crop that has not attracted international sympathy (Asogwa, Ndubuaku and Mokwunye, 2008) [8]. It is sometimes referred to as an orphan crop, as most countries outside Africa, and Africans to an extent, shy away from kola production and improvement (Asogwa, Ndubuaku and Mokwunye, 2008) [8]. Thus the geographical and chronological spread in trade and use of kolanut has inevitably created a high demand for kolanuts far in excess of its production (Anon, 1985).

Nigeria accounts for about 70% of the total world production of kola nuts [2]. About 90% of the kola produced in Nigeria is consumed within the country while 10% is exported. Hundreds of tonnes of dried kolanuts are exported annually from West Africa to North America and Europe which are used for the preparation of beverages. Kolanuts have great socio-economic importance because of their applications in native ceremonies like marriages, child naming, and installation of chiefs, funeral and sacrifices made to the various gods of African mythology (Nzekwu, 1961; Daramola 1978 [6] a Opeke 1982) [18]. In Nigeria, it has commercial value in manufacturing of pharmaceutical products (e.g. laxatives) the presence of alkaloids and other chemicals in kolanuts such as caffeine, kolatin, the obromine and theophylline make them suitable for use in drug preparations (Anon, 1985; Olunloyo, 1979). The kola pod husk has also been utilized for the production of liquid soap (Yahaya*et al*., 2001). Kolanut has been used as a base for new brand of chocolate and wine (kola chocolate and kola wine) which were developed by CRIN researchers (Famuyiwa, 1987). Eka, (1971) also reported the possible use of pulvenizedkolanut for the preparation of hot non-alcoholic beverages. The roots provides excellent chewing sticks for cleansing the teeth and the wood is used in local carvings, coach work and boat building (Nzekwu, 1961). In Nigeria, despite the industrial values of kolanut, it is often chewed individually or in group settings as stimulants or for mental alertness in order to remain awake for a longer time due to presence of these ingredients caffeine, theobromine, kolatin which prevents sleep, thirst and hunger and also acts as an anti-depressant (Opeke, 2005) [18] and thus has enhanced its continuous use for about 1000 years now in Nigeria (Agatha *et al*., 1978., Arogba, 1999). High consumption of kolanut has also been reported to have some negative consequences like high blood pressure, insomnia or ulcer in pregnant women and being carcinogenic due to its high nitroso compounds (use internet) content (Artfield, 1985 and Ajai *et al.,* 2012). The storage of kolanuts has being a laborious and delicate task. Though much of the produce is chewed in its fresh state, yet the bulk of the kolanut is harvested once in a year. Thus preserving the freshness of the nuts for many months without storage moulds and the attack of kolanut weevil have shown a great challenge which farmers and traders seek to solve. With a poor storage method, loss of up to 50% per tonne due to storage moulds may be recorded according to farmers. Often control of spoilage is done by removing the infested kolanuts at intervals during the storage period (Agbeniyi, and Ayodele, 2010). The kolanut weevils *Balnogastris kolae* and *Sophrorhinus spp*. are the most destructive field-to-store pests of kolanuts in West Africa (Daramola, 1978) [6]. The weevils are capable of causing 30-70% damage on the stored kolanuts while 100% damage has been recorded in cases of late harvest (Daramola, 1973) [7]. Adults of *Balanogastris kolae* is the most common and economic important weevil of kolanut, they are usually dark brown; 3-4mm in length and 1.5-2mm wide. The female lays eggs inside the nut about 1cm deep and in other parts of the fruits through wounds and holes made by other insects (primary insect pest) such as *Ceratitis colae* Silv. or through cracks on the husk created when the follicles dehisce before harvest or during processing. Incubation lasts for about 4-6days. Larva stage takes 17-20days and the larva feeds extensively on kolanut reducing it to brown powdery mass. Pupation lasts for about 5-6days. The larval and pupal periods takes place inside kolanut. The average period from oviposition of the emergency of the adult of *B. kolae* is 29days and 31days for *Saphrorhinus*spp. with the oviposition starting on the third day (Daramola, 1973, 1987/ 78). Breeding continues throughout the year on left over kolanuts and nut produced in-between the main harvest seasons (Alibert and Mallamaire, 1955 [10]; Daramla, 1974). In an attempt to protect stored kolanuts from the attack of weevils, kolanuts farmers and traders use various types of pesticides including banned ones (Asogwa; Ndubuaku and Mokwunye 2008 [8], Paul, Oduwole and Adebiyi, 2013) [17]. Some of these insecticides include Chlordane and endosulfan (Paul, Oduwole and Adebiyi, 2013) [19]. Chlordane was the first cyclodiene insecticide to be used in agriculture and was the second most important organochlorine insecticide in the United States in 1976-77 (Nomeir and Hajjar. 1987); while Endosulfan is a pesticide belonging to the organochlorine group of pesticides, under the Cyclodiene subgroup (Hermann, 2003) [9]. The applied pesticides in their characteristic nature have the ability to permeate plant cells and remain as residues. Several research works have reported the presence of pesticide residues in various foods, vegetables, soils, sediments and diverse environmental matrices. Studies/Study has have shown that, 50% of kolanuts samples obtained from Oyo State, 70% from Ogun State and 100% from Osun State contain chlordane residue while all the samples from Oyo Ogun and Osun States had endosulfan residues (Paul; Oduwole and Adebiyi, 2013) [17]. There is experimental evidence of adverse effects of endosulfan on the male reproductive system, delaying sexual maturity and interfering with the sex-hormone synthesis (Habibullah*et al*., 2003). Endosulfan is a proven to be endocrine disruptor [Soto;Chung and Sonnen, 1994] and has potential to induce hypo thyroidism [Anon PAN Pesticide Database 2001] [3]. It competes for estradiol for binding to estrogen receptors, thereby inhibiting hormonal function (Grumfeld and Bonefeld-Jorgensen 2004). It harms the reproductive system by affecting quality of semen, sperm count, spermatogonial cells, sperm morphology and other defects in male sex hormones (Pandey *et al.,* 1990). Thus, the specific objective of this study is to evaluate the effect of varied moisture content of kolanut on *B. kolae* as an alternative method of storage to reduce economic loss and as well ensure healthy consumption of kolanut.

Materials and Methods

Fresh kolanuts (*Cola acuminate* and *C. nitida*) and infested nuts that served as source of infestation (Oladokun, 1982) [14] (Figure:1-3) were procured from two different places; Ojaoba markets in Ibadan, Oyo State and Ndioro market, Umuahia, Abia State of Nigeria. Eight perforated plastic custard buckets (width 19cm and length 18cm) and two wooden netted baskets (width 24cm and length 22cm) were used as storage materials.

Fig 1: Fresh uninfested *Cola nitida* (red and white colour).

Fig 2: Fresh uninfested *Cola acuminate* covered with testa

Fig 3: Infested kolanuts as source of infestation

Percentage moisture content

Percentage Moisture content was determined using dried to constant weight method in an air oven at 105°C for 15 hours. The percentage moisture content was calculated (Agrawal, 1980; AOAC**,** 1990) using the formula; Moisture content = Initial weight - Final weight

$$\text{Percentage moisture content (\%)} = \frac{\text{Moisture content x 100}}{\text{Initial weight}}$$

The initial weight of each set up was taken and weekly weight measurement of the set up after infestation was also recorded. The data collected was analyzed using descriptive statistics and analysis of variance (ANOVA) (Ndagi *et al.*, 2012).

Assessment of kolanut weight loss

Four sets per species of kolanut containing 25 nuts were set up using the storage materials and each set was labeled A, B, C and D. Furthermore, each set was infested with 20 unsexed *B. kolae.* The control setup containing same 25nuts was also placed. The setup was stored in the laboratory for a period of 11 weeks for observation. Each set up was reweighed consecutively every 7 days (1 week) interval to assess weight loss until the 11th week.

The Biology of (*Balanogastris kolae*) kolanut weevil

Eighteen kolanut each of *Cola nitida and C. acuminate* was placed into a plastic storage container in three replicates namely sample A, B, and C. Five adult *B. kolae* in the ratio of 2 males to 3 females was introduced into it each set up. After two weeks of set up, the parent insects were removed. This set up was left for 28days and was monitored weekly for progeny emergence and to compare emergence rate in the two variety of kolanut by using 3 nuts weekly from each sample as destructive sample.

Results

Percentage moisture content

Percentage Moisture Content of *C. nitida* and *C. acuminate* Seeds Table 1 shows the percentage moisture contents of *C. nitida* and *C. acuminate* seeds at temperature of 25 ± 5°C and relative humidity of 70 ± 5%.The moisture contents of *C. nitida* and *C. acuminate* seeds were 53.10 ± 2.30% and 55.20 ± 3.40% respectively.

Table 1: Percentage Moisture Content of *C. nitida* and *C. acuminate* Seeds

Seed	Moisture Content (%)
C. nitida	53.10 ± 2.30
C. acuminate	55.20 ± 3.40

Table: 1 showed that *C. acuminate* has a higher percentage moisture content of 55.20% compare to the *C. nitida* which has percentage moisture of 53.20% but not significantly different (p>0.05). This level of moisture content in *C. acuminate* was as well observed to favour early emergence of *Balanogastris kolae* larvae during it life cycle and a reason for higher mean weight loss due to infestation with *B. kolae* during storage. This finding supports the work of Esther *et al*., (2010) that moisture loss in stored kolanut is responsible for nut loss.

Percentage weight loss

Weight loss and nut damage due to infestation varied significantly (p<0.05) with percentage moisture content and variety of kolanut (see table: 2 and 3). *Cola acuminate* was the most damaged variety while *C. nitida* was less damaged. Among the two varieties (see Table:4) sample A of *C. acuminata* showed a higher weight loss of 68.83 g (%) while *C. nitida* had 54.25g (%) weight loss both in 8weeks of storage and in control experiment, 85.90g (%) higher weight loss occurred in *C. acuminata* compared to 78.37g (%) weight loss in *C. nitida* that was recorded in 10 weeks for each though not significantly different (p>0.05) considering the time interval it took the both variety to gradually attain final weight (see Table:5).The caffeine content of kola differs within species and kolanuts variety and this reduces level of infestation (Esther *et al*., 2010). According to Asogwa *et al*., 2012, *Cola nitida* is preferred in international trade because of its high caffeine content and the white strain is most valued. This suggests why *C. nitida* had less damage and lower moisture content when compared to *C. acuminata*.

Table 2: Mean weight loss in *C. acuminate* at Eleven (11) weeks storage period

C. acuminata	**Weight before Infestation**	**Range of weight loss (g)**	**Mean ±S.E±±±**
control	478.99	0.02 -177.11	43.14 ±16.82^{a}
Sample A	417.17	0.00 - 118.42	35.89 ± 15.86^{a}
Sample B	376.69	24.91 - 50.54	37.23 ± 4.45 ^{a}
Sample C	354.34	13.65 – 44.61	29.99 ± 7.37^{b}
Sample D	336.40	10 – 12.05	11.00 ±1.00^{b}

Mean followed by the different letters in columns are significantly different (*p*<0.05). Using Paired Samples Test.

Table 3: Mean weight (g) loss in *C. nitida* at Eleven (11) weeks storage period

C. nitida	**Weight before Storage**	**Range of weight loss (g)**	**Mean ±S.E±±±**
Control	854.25	0.00-151.68	66.95± 15.74^{a}
Sample A	735.76	10.22-88.20	49.90± 11.11^{b}
Sample B	688.18	43.24-81.77	63.42± 6.65^{a}
Sample c	550.41	24.48-76.32	49.03± 12.98^{b}
Sample D	520.10	11.10-50.99	31.05± 19.95^{b}

Mean followed by the different letters in columns are significantly different (*p*<0.05). Using Paired Samples Test.

Larvae and adults emergence of *balanogastris kolae*

After one week of study, larvae emergence was observed in *C. acuminate a*but occurred in *C. nitida* at week 2 (Figure: 4). *C. nitida* had a higher total number (105) of emerged larvae while in *C. acuminate* 57 emerged larvae were recorded. At week 3 emergence of any stage was not observed from the destructive samples used from both variety, this was pupal stage of the insect. Adult emergence (Figure: 5) was observed in week 3 of the study for both *Cola species* (Table: 4). From both the infested and uninfested kolanuts, a total of 11 adults of *Sophrorhinus spp.* emerged from them. The emergence of *S. spp.* also corroborates previous observation made by Alibert and Mallamarie, (1955) [10] and Albert *et al*., (1992) that the geographical distribution of some of the weevils is widespread and all the kola trees in Africa are believed to be infested. Woodlouse *Porcellio scaber* (Figure: 5) was found to be a natural enemy of *B. colae*, feeding on their developmental stages.

Fig 4: Emerged kolanut weevil larvae *Balanogastris kolae*

Fig 4: Emerged young adult of *Balanogastris kolae*

Fig 5: Woodlouse *Porcellio scaber,* natural enemy of kolanut weevil

Table 4: No Emerged Larvae and Adults *Balanogastriscolae* (Per 3 Seeds of *Colaacuminate* and *C. nitida*)

Variety1 *C. acuminate*	**WK1**	**WK2**	**WK3**	**WK4**	**WK5**	**WK6**
	No of Larvae	No of Larvae	No of Adult	No OF Adult	No of Adult	No of adult
Sample A	4	2	0	0	0	16
Sample B	5	24	0	3	78	47
sample C	0	22	0	0	95	33
Total	9	48	0	3	175	96
variety 2 *C. nitida*						
Sample A	0	18	0	0	43	93
sample B	0	38	0	0	88	96
sample C	0	49	0	2	81	35
Total	0	105	0	2	212	224

Discussion

The farmers and kola vendors are in the habit of adding Gamalin 20EC (Organochlorine insecticide that has been banned) and other synthetic insecticides to the water for soaking fresh *Cola acuminate* or *cola nitida* during primary processing. They usually add between 10-20mL to a bowl of water (20-30L) for soaking a basket of fresh nuts depending on the size of the basket. The chemicals are readily available in the kola markets, where it is hawked freely in small quantities at affordable prices. The farmers who have resorted to this practice to practically reduce the menaces of weevil infestation on stored kolanuts as untreated nuts deteriorates within 3-4weeks. Unfortunately, this act is undesirable as kolanuts does not undergo any other formal processing before consumption. Thus, there is an urgent need for an atlernative means of protecting kolanuts from the weevil to be proffered and transferred to the kola farmers and merchants so as to save kola consumers of an impending calamity and as well reduce the loss encured by farmers due to high level of infestation. Though there was no significant different in the moisture content of the *cola spp.*, moisture content range of 40-60 have been reported to favour oviposition and development of the kolanut weevils in the absence of an adequate control measures, especially during the early stages of the weevil **(**Esther *et al*., 2009, Kareem, Owolarafe and. Ajayi, 2013). This has led to high significant level of weevil damage on stored kolanuts thus, encouraging continuous development of various instar stages of the kola weevil within field infested nuts (Daramola, 1973 [7]; Ivbijaro, 1977; Ojo, 1979). From the result obtained, it was observed that *B. kolae* bore nuts of *C. acuminata* faster and may as well explain why traders and farmers do not remove the testa of *C. acuminata* after processing the nut. The caffeine content of kola differs within species and kolanuts variety and this reduces level of infestation (Esther *et al*., 2010). According to Asogwa *et al*., 2012 [8], *Cola nitida* is preferred in international trade because of its high caffeine content and the white strain is most valued. This suggests why *C. nitida* had less damage and lower moisture content when compared to *C. acuminata*. According to Kareem. Owolarafe and. Ajayi., (2013), increase in moisture content of *C. nitida* ranging from 47.48 to 61.4% affect some physical properties of kolanut such as the mean length, width thickness geometric mean, diameter, surface area and volume. Moisture content of the nuts can be reduced by curing process during which considerable “sweating” occurs on the nuts(Asogwa *et al.*, 2012a, b, c) [8]. Early emergence of *Balanogastris kolae* larvae in *C. acuminate* was as a result of its higher moisture content *unlike in C. nitida.* The emergence of *Sophrorhinus spp.*, showed that the geographical distribution of some of the weevils is actually widespread and all the kola trees across Africa have high chances of being infested.

Conclusion

The study has shown that, *B. kolae* bore and feed extensively on kolanut with high moisture compare to those with reduced moisture content, although both fresh nuts and nuts with reduced moisture content were also bored but it was at different level of infestation.

Despite the fact that, dry to constant weight method of storage will encourage longer storage which will be useful for textile companies and for other industrial uses, it was also observed that other stored product insect pest

(*Tribolum. Spp.*) Infested these kolanuts even when properly dried. Hence there was an indication that reduced moisture content of kolanut helps to lower rate of infestation by *B. kolae* in storage and it is more cost effective and safe without pesticides residue which can pose serious health threat to consumers. The study also showed that, *B. kolae* bore and feed extensively on kolanut with higher moisture. Though both fresh kolanuts and kolanuts with reduced moisture content were bored but it was at different level of infestation. Continuous dryness of the pest in storage may encourage longer storage period which will be useful for end users during product off season. Hence there was an indication that reduced moisture content of kolanut helps to lower rate of infestation by *B. kolae* in storage and it is more cost effective and safe without pesticides residue which can pose serious health threat to consumers. In studying the biology of *B. kolae*, the least period of emergence of *B. kolae* from oviposition to adult stage was observed to between 1- 4 weeks in both kolanut varieties and level of moisture content influences progeny emergence.

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