

**ASSESSMENT OF TWO PLANT-BASED MATERIALS AS
ALTERNATIVE ATTRACTANT OF HONEYBEES (*APIS MELLIFERA
ADANSONII*) FOR PRODUCTION OF HONEY AND OTHER HIVE
PRODUCTS IN AWKA**

Comment [Rev.1]: Readers in Europe probably don't know where Awka is, please add (Nigeria)

Abstract

Aim: Honey is the earliest sweetener human beings have found. The values of honey are too numerous, such as food source, raw material for industries and as a symbol employed in therapeutic ceremonies, but its availability and supply through traditional honey hunting remain a major constraint to its production and supply. This field trial assessed the efficacy of Aidan fruit (*Tetrapleuratetraptera*) extract and Pineapple juice as alternative baits of honeybees (*Apis mellifera adansonii*) for the production of honey and other hive products.

Study Design: Completely Randomized Design.

Place and Duration of Study: Honeybee Research Centre of the Department of Zoology, Nnamdi Azikiwe University, P.M.B.5025, Awka, Anambra State, Nigeria, between July 2020 and February, 2022.

Methodology: Phytochemical analyses of the two plant-based materials were conducted in the laboratory using standard scientific methods. A total of 9 hives

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divided into 3 treatment groups: A₁₋₃, P₁₋₃ and H₁₋₃ were used for the study. All the experimental hives were installed at the apiary unit of the HRC and monitored daily until colonization occurred.

Data collected were subjected to Analysis of Variance (ANOVA) while sample means were separated using Duncan's Multiple Range Test at 5% significant level (P = 0.05).

Results: The results revealed that the trial baits contained moderate (++) presence of terpenoids as phytochemical component responsible for attracting bees. The results revealed also that the hives treated with the trial baits recorded equal rates of colonization of 66.67% as against the control which recorded 100% respectively.

Conclusion: The results suggest that these two plant-based materials can serve as alternative attractants of honeybee in beekeeping for the production of honey and other hive products.

Keyword: Honey; Aidan fruits; Pineapple; Terpenoids; Honeybees; Attractant.

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1. INTRODUCTION

Honeybees are one of the most economically beneficial insect known for honey production and pollinating activities [1]. According to [2]honey is a sweet edible liquid and one of the best blessings of nature which is made available to us by honey bees from nectar. [3]observed that 200g of honey is equivalent to 1.135kg of milk or 340g of meat or 10 eggs and with milk form a perfect meal.

Comment [Rev.4]: please add other references as well

Comment [Rev.5]: some authors [2]

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According to[4]annual world consumption of honey shows that 90% are eaten directly as honey, while the remaining 10% are used for various commercial and domestic products.The values of honey are too numerous that honey production and supply through traditional honey huntingof wild bee colonies in hollow cavities in trees or rocks, with annual production of about 2,800 tones cannot commensurate with honey demand in Nigeria[4]. Low domestic supply of honey and honey products which cannot meet the local demand in Nigeria has left the country with no option but to import honey from other countries such as China, America, Canada and India which is very expensive and has posed a great challenge to beekeepers and researchers in Nigeria.There is definitely no honey production without honeybees. Attracting wild colonies honeybees into the bee hive is a key step in the beekeeping.

Presently, farmers find it much cost intensive using honey or beeswax to attract bees into the hives, hence they face a major problem of attracting bees to colonize the hives and this initiates a search for alternative attractants. To ensure the availability of honey, other plant-based materials that are cost effective and eco-friendly can be assessed for their attractant potentials. Therefore, the need arises for field assessment of these two plant-based materials as alternative baits to attract honeybees to boost production of honey and other hive products in Awka.

The aim of this research is to assess two plant-based materials as alternative baits of honeybees, for production of honey and other hive products in Awka.

1.4 Objectives of the study

The objectives of the study were to:

- i. determine the phytochemical composition of the bait types.
- ii. determine the effect of the bait types on colonization of African honeybees
- iii. determine the effect of the bait types on colony productivity of African honeybees.

1.5 Aidan Fruit: Benefits, uses and Phytochemical composition

Aidan fruit (*Tetrapleura tetraptera* Schumach. and Thonn) Taub, Mimosaceae is a deciduous plant which is usually located in the lowland rainforest of tropical Africa, particularly in Western African countries such as Cameroon, Ghana and Nigeria. It is a leguminous tree belonging to the family Fabaceae. It is believed to have nutritional and therapeutic properties, which emphasize its popularity in many African homes [5]. In Nigeria, Aidan fruit popularly known as Osakrisa or Oshosho in Igbo, Aridan in Yoruba, Dawo in Hausa dialect is highly sought after because of its highly nutritional and pharmacological properties [6]. The fruit is known to have a characteristic pungent and aromatic fragrance which could be responsible for its insect repulsive features [7]. In orthodox practices, soup and porridge made out of aidan fruit is used to prevent post-partum contraction [8][9] and also to foster lactation in nursing mothers [10] as cited by [6]. It has also been reported that extracts from the fruit has been used traditionally in the management of convulsion, rheumatism, leprosy and certain inflammations [8]. Nutritionally, proximate analysis has shown that aidan fruit is rich in carbohydrate, crude fibre [11], fats and oil, crude protein, ash and moisture [6]. Researchers have also reported that the fruit is rich in Vitamins A, C and E [5]. Researches on phytochemical composition report that aidan fruit contains in appreciable quantities saponin, tannin, steroids, terpenoids in very high amount, alkaloids,

Comment [Rev.7]: Correct: *Tetrapleura tetraptera* (Schumach. & Thonn.) Taub.

Comment [Rev.8]: Incorrect! *Tetrapleura tetraptera* is a species of flowering plant in the family Fabaceae native to Western Africa. You probably thought about Mimosoideae subfamily or more correct Mimosoid clade??

Comment [Rev.9]: Add references

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flavonoid, resin in relatively high concentration while glycosides in very minute concentration [6];[11].

1.6 Pineapple: Benefits, Uses and Phytochemical composition

Pineapple (*Ananas comosus* L.), belonging to the family Bromeliaceae is known to be one of the most important commercial fruit crops globally. It is native to central and South America. It is grown in several tropical and sub-tropical countries including Hawaii, India, China, Kenya, Nigeria, South Africa, Malaysia, Philippines and Thailand [12]. It is also referred to as the queen of fruits on account of its excellent flavour and taste [13]. Pineapple is the third most important tropical fruits in the world after Banana and Citrus [14]. It is still a true exotic, as it is a member of the bromeliad family of which are composed of rare edible fruits. This fruit is highly perishable and seasonal. It is known to have very nutritional values as mature pineapple fruit contains about 14% of sugar; a proteolytic enzyme, and an appreciable amount of citric acid, malic acid, vitamin A and B [15]. Pineapple contains considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fibre, water and different minerals that is good for the digestive system and helps in maintaining ideal weight and balanced nutrition. It has been observed through research to contain minimal fat and sodium [16]. Investigations on the composition of pineapple have most times been focused on the edible part of the plant. [17] and [16] stated that the edible parts of pineapple

contains 81.2 to 86.2% moisture, and 13-19% total solids, of which sugar (sucrose, glucose and fructose) are the main components. Carbohydrates compose up to 85% of total solids whereas fibre makes up for 2-3%. Considering the organic acids present in pineapple, it was also observed that citric acid is the most abundant. Very low ash content, nitrogenous compounds and lipids (0.1%) were recorded in the pulp of pineapple. Also, about a quarter percentage of nitrogenous compounds are true proteins. Out of this proportion, about 80% has proteolytic activity due to a protease known as Bromelin. Pineapples are consumed or served fresh, cooked, juiced and can be preserved. Fresh pineapple contains other minerals such as Calcium, Chlorine, Potassium and Phosphorus [17], [16].

Pineapple may be consumed fresh, canned, juiced, and are found in a wide array of food stuffs such as in desserts, fruit salad, jam, yogurt, ice cream, candy, and as a complement to meat dishes [14], [18]. In Nigeria, it is used in smoothies, eaten fresh or used in pineapple juice, however, pineapple does not lend itself well to freezing, as it tends to develop off flavours[16]

Phytochemical screening of pineapple showed presence of alkaloids, flavonoids, saponins and tannins in the leave extract [18]. Quantitative screening of some extracted phytochemical show that the extracts of pineapple peel contained alkaloids, oxalate, tannins, phytate and glycosides. [19] and[20] also recorded that

phytochemical screening of pineapple fruit shows it contains alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids and phytosterols.

MATERIALS AND METHODS

2.1 Study Area

This research was carried out at the HoneyBee Research Centre (HRC) of the Department of Zoology, Nnamdi Azikiwe University, Awka for a period of fifteen months. Awka is the capital of Anambra State. Awka lies within coordinates $6^{\circ}12^1\text{N}$ and $7^{\circ}04^1\text{E}$ [21] while the HoneyBee Research Centre of the Department of Zoology lies within E: 291046.427, N: 691554.263 [1]

2.2 Experimental Design

The experiment followed the completely randomized design. It was carried out on Modified Wooden Top bar hives. The beehive comprised of the bottom board, main cover board, brood chamber as stated by [22]. A total of 9 hives divided into 3 groups were used for the study. First group labeled A_{1-3} were baited with Aidan fruitpaste and the second group of the hives labeled P_{1-3} were baited with Pineapple juice while the third group labeled H_{1-3} baited with honey served as standard bait.

2.3 Procurement of research materials

Honey used for the experiment was obtained from Honeybee Research Centre (HRC) of the Department of Zoology, Nnamdi Azikiwe University, Awka.

Aidan fruits and Fresh Pineapple fruits were purchased from the market and identified and authenticated at Department of Botany Nnamdi Azikiwe University, Awka.

2.4 Hive treatment and installation

In the apiary, each of the hives were cleaned, baited and placed at random within a distance of 4-10 metres from each other depending on shades and other physiognomic conditions.

Comment [Rev.11]: meters

2.5 Analysis of Aidan fruit

The fruits were analyzed for presence of phytochemical compositions at the Biochemistry Laboratory, Nnamdi Azikiwe University, Awka and the Research, Industrial and Analytical Laboratories, Onitsha, Anambra State.

2.5.1 Preparation of attractants

2.5.1.1 Preparation of Aidan Fruit Bait

Samples of Aidan fruits (50 g) were also taken and soaked in water for at least 72 hours with regular agitations done twice daily according to the method described in

[23] to soften and break the cell plant walls to release the soluble phytochemical properties.

2.5.1.2 Preparation of Pineapple fruit bait

Fresh Pineapple fruits were purchased from the Eke Awka market, with the bark removed and the succulent fruit blended into juice.

2.6 Phytochemical Analyses of Bait types.

The qualitative phytochemical analyses of the plant extracts was carried out using the following standard methods [24][25][26][27][28][29][30][31][32]

2.7 Data Collection

2.7.1 Determination of the effect of the bait types on colonization rate of African honeybees

Colony establishments on the three hive treatments labeled A, P and H including A₁₋₃, P₁₋₃ and H₁₋₃ were monitored for one hour, between 9am to 10am twice a week for 19 months (July, 2020 to February, 2022). The number of hives colonized and the date of colonization were recorded. The colonization rate per treatment was expressed in simple percentages using the formula in [33].

$$\text{Percentage colonization} = \frac{\text{Number of colonized beehives}}{\text{Number of beehives installed}} \times 100$$

2.7.2 Determination of the effect of the bait types on colony productivity

Colony productivity (honey yield and Beeswax) of the different hive treatments were assessed. The colonized hives were checked for honey formation and subsequently, the hives with ripe honey combs were harvested four times during the course of the study. The periods of harvest were November 2020; February, 2021; November, 2021 and February, 2022 following the floral calendar of honeybees and beekeeping seasons [34]. Honey from hive treatment H₁₋₃, A₁ and A₃, and then P₁ and P₂ were harvested and processed using the method described by [34].

After extraction of honey, the crushed combs were processed for beeswax using [34] method. The extracted beeswax was weighed and the weight recorded, and later packaged in container.



Plate 1: Ripe honey comb



Plate 2: Honey harvested

2.8 Statistical Analysis

Data collected on photochemical analyses were presented using simple descriptive and qualitative methods. Data on hive colonization were calculated as simple percentages. Data on hive colony productivity were subjected to Analysis of Variance (ANOVA) while sample means were separated using Duncan's Multiple Range Test at 5% significant level ($P < 0.05$). Microsoft Excel, 2016 was used to plot the graphs.

Comment [Rev.12]: What programs did you use for statistical analysis? Statistica, Mathematica, R?

RESULTS

3.1 Phytochemical composition of the bait types

The result of phytochemical composition of the trial bait materials revealed the presence of proteins, triglycerides, phytosterol, phenols, steroids, flavonoids, terpenoids, vitamins, folic acid and simple sugars. The results of the important phytochemical components (terpenoids) necessary for attracting bees are presented in Table 1.

Table 1: Phytochemical composition (Qualitative) of the different bait types

Bait Type	Terpenoid composition
Honey	+++
Aidan	++
Pineapple	++

+++ = Strong ++ = Moderate + = Weak

3.2 Effects of Bait Types on Colonization rate of African Honeybees

The overall results of colonization in this study are represented in Table 2.

The effects of different baits in the overall colonization rate of the African honeybees were 77.78%. Hives baited with Aidan fruit and pineapple had a colonization rate of 66.67% while hives baited with honey had a 100% colonization rate. The result shows no significant difference between the colonization rates of the bait ($P > 0.05$).

Comment [Rev.13]: colonization?

UNDER PEER REVIEW

Table 2: Effect of bait types on hive colonization by colonies of African honeybees at various months

Baits	No. installed	Months and number of hives colonized																		Total	Colonization (%)
		2020												2021							
		Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov			
Honey	3	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00
Aidan	3	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	66.67
Pineapple	3	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	66.67
Total	9	1	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	77.78

3.3 Effect of bait types on Colony productivity of African Honeybees

3.3.1 Effect of bait types on Honey Yield

Figure 1 shows the mean quantity of honey harvested from hives baited with the different bait materials. Hives baited with honey produced 4.37kg, 2.82kg in hives baited with pineapple and 1.68kg in hives baited with Aidan fruit. There was significant difference at ($P < 0.05$) in the mean weight of honey harvested among the different hive treatments.

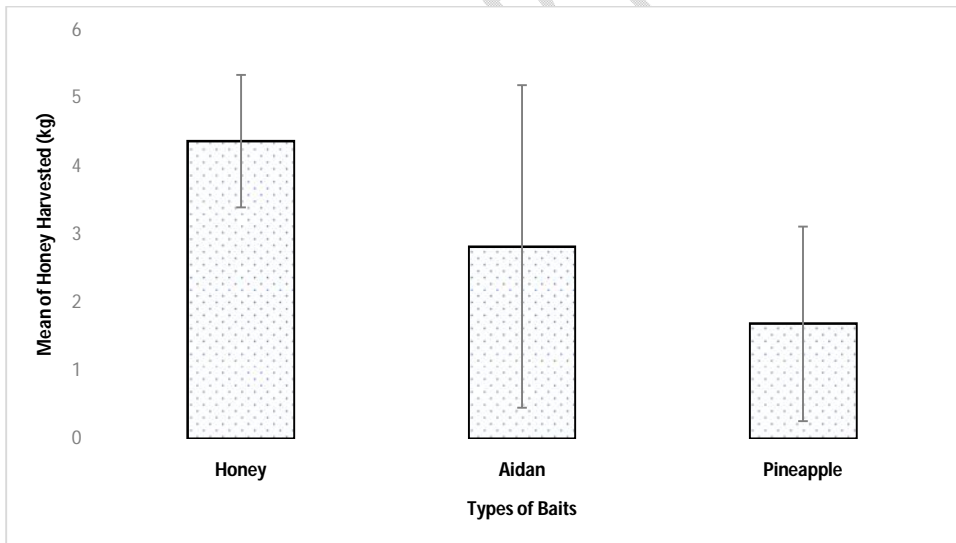


Figure 1: Effect of Bait Types on Honey Harvest

3.3.2 Effect of bait types on Beeswax Extracted

Figure 2 presents the mean quantity (1.38kg; 0.65kg; 0.88kg respectively) of beeswax extracted from the different hive treatments (H₁₋₃, A₁₋₃ and P₁₋₃). There was no significant difference ($P>0.05$) in the quantity of beeswax extracted among the baits.

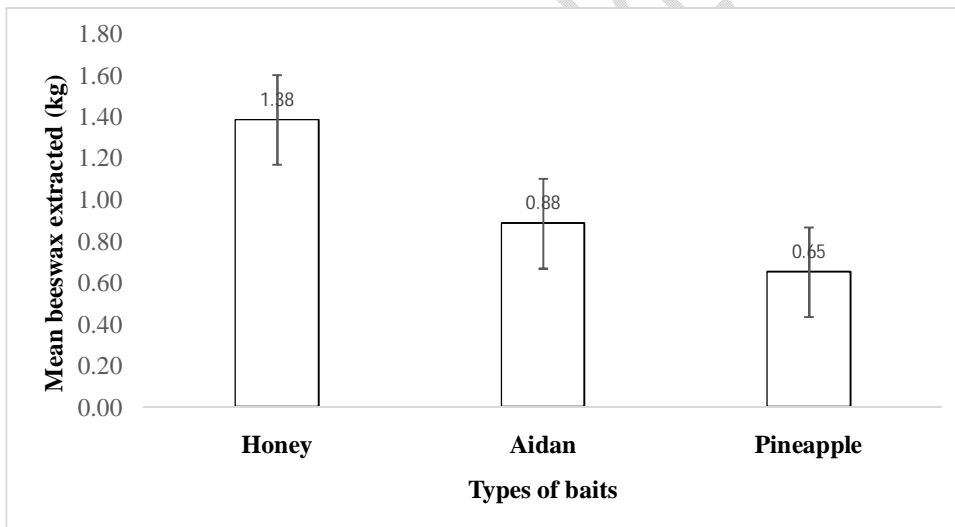


Figure 2: Mean Quantity of Beeswax Extracted

DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 Discussion

Results of phytochemical analysis of the two assessed baits revealed presence of moderate (++) amount of terpenoids. The findings of the phytochemical screening of honey agrees with those of [35] which revealed that honey samples are composed of tannins, phlobatanins, flavonoids, terpenoids, glycosides, saponins, alkaloids and fluorides. The findings of [36] and [37] agreed that honey contains essential phytochemicals such as flavonoids, tannins, phenols, terpenoids and saponins. Also, results of phytochemical screening of Pineapple juice agreed with those of [20] who recorded that phytochemical screening of pineapple fruit shows it contains alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids and phytosterols. It was also in agreement with the findings of [18] which showed presence of alkaloids, flavonoids, saponins and tannins in the leave extract in pineapple juice. Similarly, the phytochemical components of Aidan fruit was in agreement with those of [6] and [11] who reported that Aidan fruit contains in appreciable quantities saponin, tannin, steroids, terpenoids in very high amount, alkaloids, flavonoid, resin in relatively high concentration while glycosides in very minute concentration.

Comment [Rev.14]: saponins

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Colonization rate of the African honeybees was highest in the control treatment (100%) and moderately in the tested plant materials (66.67%, 66.67% respectively). Colonization was not significantly influenced by the bait types during the study period and this could be as a result of the same amount of terpenoid present in the two plant materials tested for their attractive potentials of honeybees for colonization of hives as in the case of this study. However, the highest percentage of colonization (100%) was recorded in the hive baited with honey. [38] noted in contrast that hives baited with beeswax recorded highest level of colonization as against honey, pineapple fruit juice, lavender, hayaki, locust beans and fresh cow dung. Similarly, [39] reported that hives baited with beeswax had the highest colonization rate (100) as against pineapple fruit (66.6%). The findings of their study also revealed that there was no significant difference between bait types which is in agreement with the results of the present study. Furthermore, [40] report is in agreement with the findings of this study that hives baited with bee wax had the highest percentage colonization (66.67%) which was not significantly different from 33.33% observed in slum gum, honey and propolis. Generally, colonization was strong (77.78%) in the study area within the study period. This could be attributed to favourable climate conditions and improved vegetation within and around the study area. This is in contrast to low colonization reported by [38] and [41] who reported low colonization in various local

governments in Kwara state which they attributed to low age level of modern beekeeping practice in the state.

Bait type significantly affected the quantity of honey yield as observed from the result. Previous studies on the effect of treatments on quantity of honey harvested by [42] reported similar observation. In this study, the quantity of honey harvested ranged from 1.20kg – 1.6kg for treatment H, 0.6kg – 1.0kg for treatment A and 0.4kg – 0.80kg for treatment P of which all fall within the 0.00kg – 2.0kg reported by [42]. The findings of this research however contradicted that of [40] which stated that Slum gum had the highest honey yield (0.7kg). This was followed by bee wax (0.6kg) and honey (0.4kg)-baited hives while the least yield was observed in propolis-baited hives (0.05 kg).

Quantity of beeswax extracted was not influenced by the type of bait. This is in contrast to the study of [42] which revealed that bait type had significant effect on quantity of beeswax extracted. In this study, hives baited with honey recorded the highest quantity of beeswax (1.38 ± 0.286 kg), those baited with aidan fruit (0.88 ± 0.697 kg) while those baited with pineapple had the least (0.65 ± 0.521 kg), this is similar to the finding of [42] on the relationship between bait material and bee wax yield where hives baited with pineapple had the least quantity of beeswax harvested.

4.2 CONCLUSION

Honeybees play crucial role in the production of honey and other hive products. They are the principal pollinators of agricultural crops and wild plants. Based on the findings of this study, the baits should be considered for effective colonization and mounting of hives should be done prior to the month of July. Aidan fruit and pineapple juice can be adopted as alternative and cheaper baits to the scarce and costly beeswax and honey used predominantly in beekeeping. Where readily available, Aidan fruit extract can be used for better colony productivity than pineapple juice. More plant materials can be checked for the presence of attractant materials and consequently, adopted as baits if they show positive results in attraction honeybees.

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