**The Use Of *Piper Umbellatum* Leaves In The Rearing Of Immature Stages Of *Bunaea Alcinoe* [Lepidoptera: Saturniidae], An Edible Larva Of The Niger Delta.**

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**Abstract**: The use of *Piper umbellatum* (L.)leaves in the rearing of immature stages of 5th instar larvae of *Bunaea alcinoe*, edible Emperor Larvae of the Niger Delta was studied for the first time in the Post Graduate Entomology Laboratory of the Department of Applied and Environmental Biology of the Rivers State University, Port Harcourt, Nigeria in 2016. The study was made at mean laboratory temperature of 24.50C and relative humidity of 90.2% in May, 2016. Leaves of *P. umbellatum* were used in wrapping the fifth instar larvae of *B. alcinoe* which could not enter into the pupal phase. A 100% pupation was achieved compared to 56.4% of control of larvae not wrapped with *P. umbellatum* leaves [n=30]. Emergence of adults was complete that produced moths with functional wings in larvae wrapped with leaves of *P.umbellatum* and mating resulted in viable eggs with high egg hatchability too. Those in the control showed emerged adults with non-functional wings and mating activity was low and where it occurred, both egg hatchability and viability were significantly low.

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**Keywords**: *Piper umbellatum*, rearing, pupae, *Bunaea alcinoe*, Fifth instar larvae.

1. **Introduction**

Emperor moth larvae are caterpillars of the of the edible Niger Delta Moth *Bunaea alcinoe*, which feed on more than eight plant species of the Niger Delta of Nigeria. Their host plants include; *Terminalia catappa*, *T. ivorensis*, *Gmelina arborea*, *Tectona grandis*, *Anthocleista vogeli*, Cassia sp, *Anarcardium occidentale* and the ornamental plant, commonly referred to as Queen of the Night. Larvae of the Emperor moths and the aforementioned plants are valuable resource plants that contribute substantially to rural economies and nutrition in the Niger Delta sub region of Nigeria. Moth harvesting serves as income source for poor rural women and children. The Emperor moths larvae and pupae provide delicacies in form of snacks and nutritious food for rural households and some urban dwellers that migrated from the forest regions of the states. Traditionally, the emperor moth larvae have been harvested for their edibility as snacks and for subsistence use in the rural circles, but currently have been found in urban markets due to the high cost of living where they are harvested and used as supplements for proteins; it contains 55.7% protein (Amadi et al., 2005) and are therefore accepted as making significant contribution to rural diets. Emperor larvae with their relatively high protein content have been used to replace meat in the rural circles of the Niger Delta uplands where it has been used to alleviate malnutrition in preschool and primary rural (upland) children where seafoods are scarce and animal proteins are expensive. Dried Emperor moth larvae of *B. alcinoe* have a relatively high crude protein content of 67.4%. Increased conservation and supplies of the larvae can address the problem of food security and malnutrition in children and pregnant women in the rural circles of different parts of Nigeria.

Many plants harbour the eggs and larval stages of *B*. *alcinoe* on their leaves, stems and branches from where they are harvested. Harvesting of the edible Emperor moth larvae are carried out mostly by women, youths and children in order to provide financial capital for food purchases in the areas where animal proteins are expensive. It helps also in providing and increasing incomes for poor rural harvesters. Harvesting of the larvae occurs mostly during the rainy seasons during the period of availability of their host plants that coincides with their time of infestation.

*Piper umbellatum* L. is a tropical shrub with many medicinal and nutritional values in different parts of Nigeria. The plant grows in the forest zones of the Niger Delta, Nigeria. A previous study by Nwauzoma and Dawari (2013) on the phytochemical properties and proximate composition of the leaves revealed a very high amount of steroid (more than 95%), little traces of tannin and alkaloid. Saponin and phenol were slightly above 10% each, and flavonoid (less than 10%). Proximate analysis demonstrated the presence of protein (20. 56%), ash (17%), high amount of fibre (55.6%), moisture (less than 10%) and small amounts of carbohydrate and lipid (Nwauzoma and Dawari, 2013).

According to Ropke *et al.,* (2006), numerous medicinal uses have been ascribed to *P. umbellatum* depending on the region. *P. umbellatum* is traditionally used in the treatment of premature babies, before the advent of modern medicine. This is done by putting the baby in a small basket lined and covered completely with *P. umbellatum* leaves, cited as personal communication in Nwauzoma and Dawari (2013). The new-born with the mother is confined to a poorly ventilated room, till the baby matures. In those days, it is common to name such pre-mature babies ‘nwanjaa-nja’, meaning ‘a child of *P. umbellatum* (Nwauzoma, et al, 2013). Some of the essential oils from the aerial parts of *P. umbellatum*have high content of β- pinene (27%), α-pinene (18%), Е-nerodiol (12%) and β-caryophyllene (10%) (Perazzo *et al,* 2005). The roots and aerial parts contain 4-nerolidylcatechol, an antioxidant, which may explain its use in the treatment of skin cancer. It is also known to inhibit the effects of the mytotoxic phospholipase venoms of vipers (*Bothrops spp*.) (Nunez *et al*, 2005).

Plants have a limitless ability to manufacture aromatic substances mainly secondary metabolites, of which less than 10% have been synthesized. These metabolites are used by plants for different purposes like defense against predation by microorganisms, insects and herbivores (Akharaiyi and Boboye, 2010). Some of these metabolites may be involved in plant odour (terpenoides), pigmentation (tannins and quinines), and flavour (capsacin) (Ogbalu, 1986; Mallikharjuna, *et al*., 2007). These defensive molecules give plants their medicinal value which is appreciated by human beings because of their great importance in healthcare of individuals and communities. *P. umbellatum* has for long being used for therapeutic purposes and food in different parts of Nigeria. However, little is known about its chemical and nutrient compositions to support its wild usage. Therefore, this study was undertaken to examine the role of *P. umbellatum* leaves in the rearing of fifth instar larvae of the edible emperor moths which are lost in their transformative pupal state resulting in low adults’ emergence.

**2. Materials and Method**

One hundred and twenty (120) mature leaves of *P. umbellatum* were harvested from Amaku-Igbodo, Etche in Rivers State of Nigeria during the rainy season of May, 2014 and taken to the Post Graduate Entomology laboratory in the Department of Applied and Environmental Biology of the Rivers State University, Port Harcourt, Nigeria. The leaves were kept in the laboratory trays of 35cm wide and 50cm in length and they were rinsed under running water to remove sand and debris attached to them. Each leaf of *P.umbellatum* was cut longitudinally from the base of the mid-vein to the tip using a pair of sterile dissecting scissors. A fifth instar larva of *B. alcinoe* (Fig. 1) harvested from *Terminalia catappa* was placed on each half of the leaf and the leaf was rolled over the larva to the end so that both ends (anterior and posterior ends) of the larva remained open for effective respiratory activities. The other half left after wrapping was still used to rewrap the larva loosely and kept in a wooden sleeve cage.

Thirty of such set-ups constituted a replicate and there were four replicates in all. Another thirty sets of larvae were allowed to burrow into the soil left in plastic buckets containing 2000g of humus soil. Replications were as in the wrapped larvae set up; these served as control. The two trials were run concurrently at laboratory temperatures of 24.5OC and a relative humidity of 90.2%. Observations continued till fifty days after for the calculation of essential biometric measurements; namely pupal weights, percentage pupation, pupal durations, adult emergence, mating, oviposition periods, fecundity, egg hatchability and number with functional wings on emergence.



Figure 1a: Fifth instar larvae of *Bunaea alcinoe* harvested from host plant, *Terminalia catappa*.

**3. Results and Discussion:**

Pupation in fifth instar larvae wrapped in leaves of *P. umbellatum* was successful (between 97.4 and 100%) in all the four replicates maintained under laboratory conditions and in the control trials (unwrapped), pupation was significantly low (Fig. 1). *P. umbellatum* has been reported to contain a very high amount of steroid (more than 95%), little traces of tannin and alkaloid. Saponin and phenol were slightly above 10% each, and flavonoid (less than 10%) which accounts for its local use in herbal medicine and nutritional purposes (Nwauzoma and Dawari, 2013). The control trials that involved larvae burrowing into the soil had most of the time resulted in low pupation and subsequent low adults’ emergence confirms previous findings by Ogbalu, 2017 and Ogbalu and Kwokwo, 2017). Some were not able to burrow due to exhaustion as they had strained themselves covering distances searching for suitable pupation sites. Depending on the types of soil, both fungal and bacterial activities within such soils affected them. Predatory and parasitic activities of some insects’ parasitoids which penetrate their intestinal systems obstruct further embryological developments, causing mortalities and preventing their complete metamorphosis. Therefore, biotic and abiotic factors affect their pupation. However, the phytochemical contents of the *Piper umbellatum* leaves promoted their growths throughout their embryonic development till adulthood. The trend was the same as in adults’ emergence with those that were wrapped in leaves showed significantly higher emergence than those that burrowed into the soil.

In earlier studies, Ogbalu, (2017) showed that types of soil in which they pupated affected pupation in a trial when they were exposed to sandy and humus soils. Bacterial and fungal activities were significantly higher in humus than sandy soils. Most of the adults that emerged from the control trials had malformed wings and the dysfunction affected their mating and oviposition in both females and males, none could locate the other for mating and hence the females had low fecundity and their adults’ longevity was shorter for those in the control; also for the obvious reason that they could not search for nectar which they utilized as food. In the laboratory, such adults died earlier than those that emerged from wrapped leaves. The adults reared from wrapped leaves significantly showed higher longevity, fecundity, adults’ weights and higher egg hatch. Results from morphometric data showed higher records in wing spans of (14.8cm in females) of emerged adults whose pupae were wrapped in *P. umbellatum* leaves and 12.4 of those not wrapped (Table 1). Data collected on mating frequency, percentage oviposition, egg hatch, pupal weights, pupal duration, adults emergence and longevity, antennal lengths, head capsule widths were significantly higher in the category of those wrapped in *P. umbellatum l*eaves (DMRT, SNK P; 0.95). Usually, in Emperor moths, the females outlived the males; most males die after copulations.

The phytochemical analyses of the *P. umbellatum* leaves showed the inherent qualities of metabolites found in the leaves of *P. umbellatum* to the embryological development of *B. alcinoe* larvae/pupae. There have been high mortalities of pupae due to fungal and bacterial attacks as well as predation and parasitism due to insects and birds especially among the domestic forms as in *Gallus domesticus* that feed on both larvae and pupae of the moth. Insects, bacterial and avian attacks occur at pupation in the soil, or at the time they are searching for pupating sites.

The use of *P. umbellatum* leaves for the protection of premature babies till they were able to develop successfully till maturity due inherent metabolites provided by the leaves has been reported (Nwauzoma and Dawari, 2013). Our findings here showed that the same metabolites not only protected the prepupa and pupae, but also provided them with bioactive compounds that have been known to provide insecticidal, antimicrobial and some physiological effects advantageous to the growing hexapods and necessary for their nutritional requirements and development. Percentage emergence of adults was higher in pupae that were wrapped in *P. umbellatum* leaves (Fig.2). Emerged adults deposited more eggs than in the control (Fig.3), indicating the availability of the metabolites and their roles in the pupal development and growths. Some medicinal plants 0f the Niger Delta and South East regions of Nigeria are enriched with secondary metabolites which are extracted from their leaves, stems or roots. These plants contain secondary metabolites (alkaloids, flavonoids, saponins, steroids, tannins and phenolic compounds), vitamins and minerals which are bioactive compounds and have been known to have antimicrobial properties (Chung et al., 1998), medicinal importance (Edeoga, *et al.,* 2003), physiological effect in animals (Edeoga and Eriata, 2001) and are widely distributed among different plant species (Tedong, et al. 2006). Ogbalu and Williams (2015) reported on the role of *Lepidagathis alopecuroides* in the treatment of breast sores caused by Myiasis flies in some rural women. Compounds with phenolic group in its structure such as tannin have pharmacological potential which is shown on its therapeutic abilities such as antimicrobial, hypoglycemic, antidiabetic (Aslan, et al., 2008) and antidiarrhoeal (Albuquerque, et al., 2010). Tannins are diverse class of phenolic compounds occurring in many plant species (Vincken, et al., 2006).

**Conclusion**

Data obtained from previous studies showed that the phytochemical screenings of *Piper umbellatum* leaves contain secondary metabolites such as saponins, alkaloids and tannins with high biological importance to living and developing stages of the Emperor moth and also they contributed to the therapeutic elements needed for the embryonic development of the maturing pupae. *P. umbellatum* leaves provided antimicrobial and pesticidal properties to the developing larvae and offered them some degrees of protection which sustained them till maturity hence pupation was high in larvae wrapped in leaves of *P. umbellatum* than those not wrapped.

Table 1. Morphometric data of *B. alcinoe* larvae reared in wrapped and unwrapped *Piper umbellatum* leaves [n=30] [24.50C; 90.2% Rh ].

|  |  |  |
| --- | --- | --- |
| Parameter | Wrapped | Unwrapped |
| Mean pupal duration (days) | 36.2 | 40.2 |
| Pupal weights [g] | 6.5-11.7 | 3.8-6.2 |
| Mean adult longevity (days) | 2.2 (males)3.5 (females) | 1.2 (males)1.5 (females) |
| Percentage with functional wings/wing span (cm) | 98.314.8 | 28.412.4 |
| Percentage oviposition | 97.5 | 57.0 |
| Mating frequency | 80.8 | 18.5 |
| Percentage egg-hatch | 86.4 | 12.7 |
| Head capsule Widths /Antennal lengths (cm) | 0.5-0.6 cm | 0.3-0.4cm |

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