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Estimation of protein in *Oecophylla smaragdina* adults and larvae

ओइकोफिला स्मरैग्डिना के वयस्क एवं लार्वा में प्रोटीन का मात्रात्मक मूल्यांकन

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सारांश

मानवों के लिए खाद्य कीट पोषण का एक उत्कृष्ट स्रोत हैं। झारखंड के वनों से आच्छादित क्षेत्रों, विशेषतः राँची, गुमला एवं सिमडेगा में लाल बुनकर चींटी (*Oecophylla smaragdina*) अपने प्राकृतिक आवास में पाई जाती है। चींटियों तथा उनके लार्वा दोनों का उपभोग व्यापक रूप से किया जाता है। इस चींटी प्रजाति का उपयोग विभिन्न उद्देश्यों हेतु किया जाता है, मुख्यतः उनके उच्च पोषणीय मूल्य के कारण। वर्तमान अध्ययन में *Oecophylla smaragdina* के नमूनों से प्रोटीन का आकलन लोवरी पद्धति (Lowry's method) द्वारा किया गया, जिन्हें झारखंड के पोखला गाँव से संकलित किया गया था। अध्ययन में पाया गया कि वयस्कों एवं लार्वा के प्रत्येक 1 ग्राम शुष्क चूर्णित नमूने में प्रोटीन की मात्रा क्रमशः 1.578 मि.ग्रा./मि.ली. एवं 2.039 मि.ग्रा./मि.ली. थी।

कुंजी : खाद्य कीट, *Oecophylla smaragdina*, पोषण स्रोत, प्रोटीन आकलन, लोवरी पद्धति, पोषणीय तुलना।

Abstract

Edible insects are an exceptional source of nutrition for humans. In the forested regions of Jharkhand, notably in Ranchi, Gumla, and Simdega, red weaver ant (*Oecophylla smaragdina*) thrives in its natural habitat. The consumption of both ants and their larvae is widespread. This ant species is utilized for various purposes and primarily due to their high nutritional value. In the current study, protein estimation was carried out using Lowry's method, of *Oecophylla smaragdina* samples from Pokla village in Jharkhand. It was found that for each 1 g powdered samples of adults and larvae, the protein content was 1.578 mg/ml and 2.039 mg/ml respectively.

Keywords: Edible insects, *Oecophylla smaragdina*, Nutritional source, Protein estimation, Lowery's method, Nutritional comparison.

1. Introduction

Insects are found worldwide and serve variety of purposes. Since time immemorial, humans have engaged in "**Entomophagy**," which refers to the practice of consuming insects. This practice has been documented across many cultures worldwide. In addition to being a rich source of protein, insects are also known for their various medicinal benefits, with thousands of edible species recognized at different stages of their life cycles (Kumar *et al*., 2023).

In India, insects such as grasshoppers, termites, beetles, red weaver ants (*Oecophylla smaragdina*), and silkworms (*Bombyx mori*) are widely consumed by humans. Insects are a vital protein source in many regional diets, offering superior nutritional value with more iron and calcium than conventional meat, as well as a complete amino acid profile that surpasses both plant-based and animal-based diet. Their nutritional composition varies according to the species, growth stage, environmental factors and the mode of nutirion. Furthermore, insect protein can be produced sustainably on an industrial scale, making it an appealing option for feeding the world's growing population while providing all of the essential amino acids for a healthy diet (Pan *et al*., 2022b).

Recently, researchers have turned their attention to insects as a possible source of antimicrobials and anti-cancer medications (Sinha & Choudhury, 2024). This interest stems from the fact that for over 500 million years, insects have lived and developed in harsh environments, creating a variety of compounds intended to combat microbial threats (Kumar *et al*., 2023).

Fabricius initially described the *Oecophylla smaragdina* species in 1775, after classifying ants based on their outward traits. Taxonomists have now revised the classification, emphasizing the unique social behaviour of *Oecophylla smaragdina* and assigning it to the Hymenoptera subfamily Formicinae. These ants are closely related to their African counterpart, *Oecophylla longinoda*, which shares similar nest-building characteristics but a different geographic range (Bolton, B. 1995; AntWiki. 2023; Hölldobler, B., & Wilson, E.O. 1990).

Red weaver ants have long been used in traditional medicine to treat various ailments, such as cold, high fever, and earaches. Conditions like scabies, malaria, toothaches, gut related disorders and high blood pressure can be effectively managed with formic acid (Kumar *et al*., 2023). Additionally, *Oecophylla smaragdina* is believed to support women's health by addressing issues related to miscarriages and childbirth. The nutritional and therapeutic benefits of *Oecophylla smaragdina*, as well as traditional entomotherapy practices among some tribes—such as making medicinal oil from crushed worker ants to treat joint pain and skin infections—warrant further investigation (Chowdhury *et al*., 2015; Kumar *et al*., 2023).

Worker ants, known for their sour taste, are referred to as "mot som" and are commonly consumed, particularly their brood (eggs, larvae, and pupae), with a preference for the queen brood. The Mishing tribe and the Ahom community in Assam enjoy red ants (*Oecophylla smaragdina)* during the Bohag Bihu festival, with a strong belief that they can help prevent illness. In Pithra village in Jharkhand's Simdega district, people collect red ants and their eggs and cook them with mustard oil, salt, and spices. Additionally, some tribes prepare them as pickles or chutneys to accompany rice and local liquor (Chowdhury *et al*., 2015; Kumar *et al*., 2023; Van Itterbeeck *et al*., 2014b).

Figure 1: Picture showing Worker Weaver Ants.

2. Materials and Methods

Fresh samples of *Oecophylla smaragdina* were collected from the market in Pokla village, Jharkhand, 835227 (latitude 22.91804°, longitude 84.950426°). After cleaning and separating the adults and larvae, they were allowed to dry in the sunlight.

The specimen samples were homogenized to increase the surface area for extraction. 1g powdered samples of each adults and larvae were used. 10 ml ethanol was added separately to both the samples (10 mL to 1 g of powdered adult and 10 mL to 1 g of powdered larvae. The mixture was incubated at room temperature for two hours to facilitate protein extraction. Subsequently, it was centrifuged at 10,000 × g for 13 minutes at 4°C to separate the protein-rich supernatant from the debris, which was then transferred to a sterile tube. Ethanol was made to evaporate at room temperature to concentrate the proteins, and 10 ml of phosphate-buffered saline (PBS), was added separately to adult and larvae samples for reconstitution, as it helps to gently rehydrate the protein while maintaining the proper ionic strength and pH. This step ensures that the protein structure remains as close as possible to its functional or native form (Ryan, Kinsella, & Henehan, 2023).

Finally, a protein assay, such as the Lowry method, was performed to quantify the extracted proteins. The Lowry protein assay is a widely used biochemical method for estimating total protein concentration due to its sensitivity and reliability.

This assay involves two sequential reactions: first, copper (II) ions bind to peptide bonds under alkaline conditions, forming a light blue complex. In the second step, the Folin–Ciocalteu reagent reacts with this complex as well as specific amino acids—namely tyrosine, tryptophan, and cysteine—resulting in the formation of a stable blue chromophore known as heteropoly molybdenum blue.

The intensity of this color, measured spectrophotometrically at 650 nm, correlates linearly with the protein concentration. Although the assay can be sensitive to interfering substances such as detergents and reducing agents, it remains more accurate than simpler methods like the Biuret test (Ma *et al*., 2023; Lowry *et al*., 1951; Sinha & Choudhury, 2024).

Figure 2: Specimen sample of adults and larvae

Figure 3: Picture showing dried sample of adults

Figure 4: Picture showing dried sample of larvae

Figure 5: Specimen sample of dried adults and larvae after homogenization.

3. Results

A standard graph was plotted and putting the O.D. values at 650 nm of the test samples (for adults 1.474 and larvae 1.829) in the obtained line equation, the protein concentration of the samples was obtained. Qualitatively, there was a noticeable increase in color intensity of the test samples (adults and larvae) as compared to the standard BSA. The protein concentration in adults, was 1.578 mg/ml whereas the protein concentration in larval sample was 2.039 mg/ml. These results indicate that *Oecophylla smaragdina* adults and larvae have significantly different protein concentrations. Specifically, the larvae exhibit a higher protein concentration compared to their adult counterparts. This result also suggests that the nutritional needs and metabolic processes of larvae require a greater protein supply to support their growth

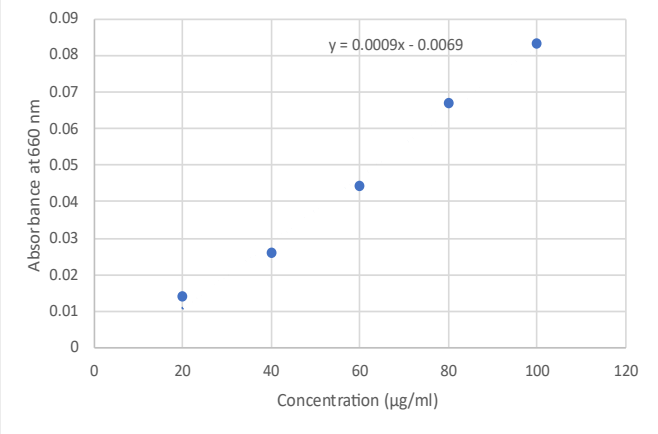

Figure 6: A graph illustrating the relationship between protein concentration and absorbance.

4. Discussion

Kumre (2024) conducted a biochemical analysis of the life stages of *Oecophylla smaragdina*, revealing that the concentrations of key biomolecules—proteins, RNA, DNA, and carbohydrates—increased progressively from the egg stage and peaked during the pupal stage. This surge in protein levels during pupation underscores the intense metabolic activity necessary for tissue remodeling and transformation in holometabolous insects. As ants transition from pupae to adults, the levels of these biomolecules decline, suggesting a redistribution of resources toward essential colony activities such as foraging, defense, and nest maintenance. This study supports the hypothesis that protein synthesis is prioritized during early development to facilitate morphogenesis, while adult ants rely more on the functional deployment of these molecules than on active biosynthesis. This developmental shift carries significant implications for understanding caste differentiation, energy allocation, and the timing of nutritional interventions in ant-based food systems.

The varying nutrient compositions at different life stages facilitate division of labor within the colony; larvae serve as nutrient absorbers, whereas adults act as energy distributors. The high nutritional value of larvae makes them a popular food choice among tribal groups, whereas adults are primarily valued for their medicinal benefits, such as the presence of formic acid, rather than their protein content. In some contexts, adults are also consumed by humans.

Recognizing the developmental and biochemical variations in edible insects is essential for optimizing harvest timing to maximize nutritional value. By harvesting during protein-rich stages, we can enhance their role as functional foods, providing high-quality proteins and micronutrients that address chronic deficiencies common in rural areas. This is particularly important for species like *Oecophylla smaragdina*, which holds cultural significance. Sustainable farming and processing not only boost nutritional benefits but also support tribal economies and promote ecological resilience. As such, entomophagy emerges as a vital pathway toward health security and food sovereignty. These insights collectively frame entomophagy not just as a nutritional solution, but as a culturally embedded, ecologically sound, and economically viable approach to enhancing tribal health security and fostering sustainable food futures. (Doloi & Basumatari, 2024; Sangeeta, G., & Gitalee, B., 2024; Kumre, S. B., 2024).

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