Research Article

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Effects of Gamma Irradiation of Egg Cells on Macro and Micro Nutrients Composition of the Edible Larval Stage of Cirina Forda (Lepidoptera: Saturnidae)

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Abstract
The need for diversification of cost-effective enrichment of human diets with macro and micro-nutrients has assumed global concerns. To this end, entomophagy has been advocated as a promising source of these nutrients but such must be predicated on the on optimization of nutritional quality of the selected insect candidates, and that informed this study. To achieve this, freshly laid eggs of Cirina forda exposed to a range of Gamma irradiation doses (0, 10, 20 and 50Gy) and subsequently raised through the edible larval stage on Vitellaria paradoxa plant's leaves. Proximate, mineral and micro-nutrient analyses of the irradiated matured C.forda larvae were carried out following standard biochemical procedures. The results of proximate analysis showed that egg exposure to Gamma irradiation significantly (P<0.05) increased dry matter and carbohydrate contents, at doses of 20Gy and 50Gy, respectively (41.43±0.01 and 21.50±0.14%, respectively), the amount of crude protein, crude fibre and ash reduced slightly in the C.forda larvae, with exposure to increasing dose of gamma irradiation (from 54.82±0.12 to 50.32±0.09, 3.93±0.11 to 0.05±0.04 and 8.50±0.07 to 6.38±0.11, respectively). The mineral analysis indicated that while, the amounts of Ca and Mn attained significant peaks in larvae whose eggs were exposed to irradiation doses of 50Gy and 10Gy, respectively (36.03±0.01 and 8.27±0.01%, respectively), Gamma irradiation significantly reduced the concentration of Mg, K, and Fe in the insects. The micro-nutrient content of the C.forda was denominated by Leucine, Arginine and Phenylalanine, with highest concentration of 7.20±0.01, 6.86±0.01 and 5.41±0.01%, respectively, and were not significantly (p<0.05) affected by gamma irradiation. These results suggest that gamma irradiation is promising in improving certain nutrition contents of C.forda and may exert a mitigating effect on anti-feedants inherent in the insect species, which need to be investigated urgently.

Introduction
The limited supply of nutrients in the diet of man in recent times has assumed the level of global concern. The increasing demand of Macro and Micro nutrients by the growing human population has attained an alerting dimension. Over 3 billion people are currently micronutrient, elements, and vitamin malnourished, resulting in egregious societal costs during learning, disabilities among children, increased morbidity and mortality rates, lower workers' productivity and high health care costs. All these factors diminish human potentials and national economic development. Nutritional deficiencies e.g of Iron, Zinc and Vitamin A etc. Account for almost two thirds of the childhood deaths worldwide [1]. Humans require at least 49 nutrients to meet their metabolic needs. Inadequate consumption of these nutrients will result in adverse metabolic disturbance, leading to sickness, poor health, impaired development in children and large economic cost on the society [2].

The limitations of over reliance on plant sources for macro and micro nutrients, and the ever increasing human demand for these elements, particularly among growing children and infants between the ages of 1-5 years, necessitates the need to search for viable alternative sources from food insects as complements and supplements for the growing human population. Therefore, the need to prevent the risk of food chain insecurity requires the development of alternative sources of proteins and nutrients for human consumption.

Insects are widely spread all over the world many of them are well known and appreciated for their characteristics [3]. Entomophagy (the habit of eating insects as food) is a well-established custom in many parts of the world and many edible insects have become food source that is being tapped by various communities in Europe, Asia, Australia and Africa [4]. People feed on different life stages of various insects. Early records showed that the ancient Greek-parthians and Nasamines ate locusts and grasshoppers as food [5]. The French feed on the abdomen of the beetle, Rhizotrogous assimilis. In Nigeria, several species of insects are prominent items of commerce in village markets and various families make fairly good living from selling insects [6]. This has contributed to the significant reduction of protein deficiencies [7]. The caterpillar of the pallid emperor moth, *Cirina forda* is of the order Lepidoptera, and family, Saturniidae. It is an insect pest of *Vitellaria paradoxa*, the shearbutter tree, and is widely accepted as a food source and is also an item of commerce in many Nigerian states [7]. The larvae of this insect are processed into the dried form and consumed as a delicacy served in snacks or as an essential ingredient in vegetable soup along with carbohydrate food in Southern Nigeria and many homes in Africa [8-10]. The nutritional qualities of edible insects have been studied by various authors and have been found to contain protein and abundant fat calories [11,12]. Ande (2002) recorded high protein content and 21.45% ether proportion in *C.forda*.

The factors hindering optimum exportation of insects as food sources are not far-fetched and they include lack of detailed knowledge of inherent macro and micro nutrients of different insect species. (Most studies on the subject are limited to macro nutrient composition at the level of proximate analysis), seasonability of availability and inaccessibility of habitats and collection sites, and poor biomass etc. The need to therefore improve the quantity and quality yields of *C.forda* especially in captivity by exposing the eggs to irradiation therefore becomes imperative. The practice of and efficacy of and safety of gamma irradiation when moderately applied to elicit somatic and germline variability in insect population and species in particular becomes a way forward. Hence this study was carried out to generate an improved biomass of *C.forda* and above all to promote entomophagy.

Materials and Methods

Source and Collection of *C.forda* Eggs

Egg clusters of *C.forda* were handpicked from the host plant, in Wuya Kanti, a small village along latitude (9^0 8'N) and longitude (5^0 49'E) in Lavun Local Government Area of Niger State about 100 kilometres from Minna - the State Capital, Nigeria. The eggs were maintained in humid boxes before transportation to the energy laboratory for irradiation with gamma rays.

Gamma Irradiation of Eggs and Laboratory Maintenance of Larvae
Three (3) egg clusters of *C.forda* in four (4) groups A,B,C,D of three (3) replicates each were tagged 0Gy (control), 10Gy, 20Gy, and 50Gy, respectively. These were tagged according to the dosages of irradiation. Gamma irradiation was done at Centre for Energy Research and Development, Obafemi Awolowo University Ile-Ife, Nigeria.

Experiment Design and Laboratory Maintenance of *C.forda*

The irradiated eggs were transported to the Department of Zoology Laboratory, University of Ilorin, Nigeria. Three egg clusters each were mounted on the stalk of *V.paradoxa* leaves with a pin, with the stalk inserted into a bottle containing borehole water to maintain the freshness of the leaves for neonate larvae feeding. This took into cognisance, the treatment groups and dosages. The eggs were observed daily for eclosion of neonate larvae in all treatments. On eclosion, the leaves were replaced daily from the *V.paradoxa* plants which abound in the University premises. Rearing of the larvae in captivity was done under laboratory ambient conditions across all the 5 instars observed in the treatment, and the six instars recorded in the control, using the standard procedures of Ande (1991).

Sample Collection and Preparation for Analysis

Fresh samples of emperor moth caterpillar larvae at maturity were handpicked from all treatments. The samples were separately washed in warm water and sundried to remove moisture. The samples were separately milled into powder to treatments ready for further analysis.

Proximate Analysis

The moisture, ash, and crude fibre contents were determined by the methods of Association of Official Analytical Chemists [13]. The total Nitrogen (%N) was determined by the micro-Kjeldahl method of (AOAC) 2000, and crude protein was obtained by using a Nitrogen protein conversion factor of 6.25. The oil (lipid%) content was obtained by extracting 5g of powdered samples with chloroform: methanol (2:1) mixture as described by the modified Folch method. The solvent was evaporated on a rotary evaporator and the oil obtained was dried in an oven at 60^0C to remove all traces of solvents. Percentage free extract (Carbohydrate) was obtained by difference applying the formula: 100% - (%Protein + %lipid + %ash + % fibre) All determinations were done in four replicates.

Mineral Analysis

Sodium (Na) and Potassium (K) were determined using GallenKamp flame analyzer, while Calcium (Ca), Magnesium (Mg), Iron (Fe), Manganese (Mn), Zinc (Zn), and Cupper (Cu) were determined using Buch 211 Atomic Absorption Spectrophotometer, including Lead (Pb) and Cadmium (Cd).

The amino acids profile was obtained using WATERS 1525 binary HPLC coupled with multi-wavelength fluorescence detector. The oil in the sample was extracted using Chloroform: methanol mixture, as described by modified method by Folch (AOAC) 2000. The extracted oil was hydrolyzed and the fatty acids converted to their fatty acid Methyl derivatives and their concentration determined using GC/MS. The defaulted samples were hydrolyzed and after derivatization, four (4) determinations were out in each of the cases.

Statistical Analysis

Data obtained from the proximate analysis of all the treatments and their replicates were statistically analysed, following standard procedures. Differences in the proximate and the mineral mean were compared using ANOVA, at 0.05 levels of significance.

Results

The effects of gamma irradiation on proximate analysis of *Cirina forda* are presented in Table 1. In certain cases gamma irradiation had significant effects on the proximate components of the insect species, and such effects were mostly dosage-dependent. To this end, crude protein, crude fibre, and ash contents were highest among the control group of insects. (U, exposed to 0Gy gamma irradiation) with mean value of 54.82±0.12, 3.93±0.11 and 8.50±0.07, respectively and lowest in the group exposed to 50gy irradiation (Mean=50.32±0.09, 0.05±0.04, 0.75±0.14 and 6.38±0.11%, respectively). On the other hand, while the 0Gy-treated insects had the highest content (58.75±0.01%), those eggs exposed to 20Gy had the least (51.37±0.01%), before increasing significantly to (55.31±0.01%) at 50Gy irradiation.

The dry matter content increased significantly from 41.43±0.01% in the control insects, to a peak of 48.63±0.01% at 20Gy treatment and there-after, reduced significantly at 50Gy treatment. The Carbohydrate content consistently increased significantly with dose gamma irradiation (range = 16.30±0.15% at 0Gy to 21.50±0.14% at 50Gy treatment). The Ether extract and organic matter components were not significantly ($P>0.05$) affected by gamma irradiation irrespective of treatments dose. While, the former ranged from 15.05±0.04% to 16.45±0.06%, the latter varied from 92.00±0.10% to 93.62±0.05%.

Table 1: Proximate composition of *Cirina forda* larvae exposed to gamma irradiation at the egg stage

Parameters (%)	Gamma Irradiation dose (Gy)			
	0 (control)	10	20	50
Moisture Content	58.57±0.01a	52.81±0.01b	51.37±0.01c	55.31±0.01d
Dry Matter	41.43±0.01a	47.19±0.01b	48.63±0.01c	44.69±0.01d
Crude Protein	54.82±0.12a	53.05±0.10b	52.80±0.12c	50.32±0.09d
Ether Extracts	16.45±0.06a	15.84±0.03a	15.47±0.03a	15.05±0.04a
Crude Fibre	3.93±0.11a	2.38±0.18b	1.95±0.21c	0.75±0.14d
Ash	8.50±0.07a	8.00±0.28b	6.53±0.32c	6.38±0.11d
Organic Matter	92.00±0.10a	93.47±0.14b	93.50±0.24c	93.62±0.05d
Nitrogen-Free Extract (Carbohydrate by Diff.)	16.30±0.15a	20.73±0.10b	23.25±0.12c	27.50±0.14d

Values are mean of three determinations ± SEM
Values carrying the same alphabet in a row are not significantly different (P>0.05).

The mineral composition, as affected by the gamma irradiation, of the insects is highlighted in Table 2. Irradiation significantly affected relative quantities of the minerals with the exception of Cadmium (Cd) which was less than 0.01% in all the treated groups. However, the mineral composition of *C.forda* was dominated Magnesium (Mg) (Range = 484.84±0.05 at 50Gy to 550.84±0.01% in the 0Gy Control group). Potassium (K) (73.85±0.01% at 50Gy to 157.33±0.015 in 0Gy Control) and Iron (Fe) (116.36±0.015% at 50Gy to 135.53±0.015% in Control 0Gy), while Zinc (Zn) and Sodium (Na) were present in moderate amounts (range = 48.28±0.005% at 10Gy to 93.11±0.01% in Control 0Gy) and 39.61±0.006 at 10Gy to 82.70±0.01% in Control respectively). Calcium (Ca) content ranged from 12.81±0.014 in 20Gy to 36.03±0.01% at 50Gy; Manganese (Mn) attained concentrations of 1.51±0.01% at 20Gy and 8.27±0.01% among the 10Gy – treated insects. Cupper (Cu), Lead (Pb), and Cadmium (Cd) were present in very low amounts of < 2.50 and sometimes as low as 0.004%. With the exception of Mn, Cu, and Ca, mineral components were significantly highest among the Control insects (i.e 0Gy) and there-after decreased with increased gamma irradiation dose. While, Manganese (Mn) and Copper (Cu) attained the highest concentration in larvae exposed to 10Gy, Ca was the most concentrated at the 50Gy treatment (36.03±0.01%). Cu, Pb, and Cd were however not detected in the insect exposed to 50Gy irradiation.

Table 2: Mineral composition of gamma irradiated Cirina forda

Parameters (%)	Gamma Irradiation dose (Gy)			
	0	10	20	50
Iron (Fe)	135.53±0.015b	130.44±0.01b	118.93±0.015a	116.36±0.015a
Zinc (Zn)	93.11±0.01c	48.28±0.005a	62.08±0.01b	58.63±0.01b
Manganese (Mn)	7.52±0.01b	8.27±0.01b	1.51±0.01a	2.25±0.01a
Copper (Cu)	0.13±0.001a	2.48±0.001c	1.25±0.0005b	Nd
Calcium (Ca)	29.38±0.01b	27.50±0.01b	12.81±0.014a	36.03±0.01c
Magnesium (Mg)	550.84±0.01c	519.07±0.01b	506.85±0.015b	484.84±0.015a
Sodium (Na)	82.70±0.01c	39.61±0.006a	36.15±0.01a	60.02±0.015b
Potassium (K)	157.33±0.015d	116.55±0.01c	81.15±0.01b	73.85±0.01a
Lead (Pb)	0.358±0.001b	0.006±0..001a	0.004±0.001a	Nd
Cadmium (Cd)	0.039±0.0005a	0.016±0.001a	0.018±0.001a	Nd

0Gy is the control group
Nd = not detected
Values are mean if three determinants ± SEM
Values carrying the same alphabet with their respective control for each parameter are not significantly different (P>0.05).

The distribution of essential amino acids in relation to increasing dose of gamma irradiation is presented in Table 3. With the exception of Cystine (range = 2.19±0.01 at 20Gy to 3.11±0.01% at 0Gy), gamma irradiation had no significant effect (P>0.05) on essential amino acids content. However, the essential amino acids component is dominated by Leucine (range = 7.08±0.01%) to 7.20±0.01%), Arginine (6.55±0.01 to 6.86±0.01%) and Phenylalanine (5.05±0.01 to 5.41±0.01%). Lysine, Histidine, Valine, and Isoluecine, were present in moderate amounts of about 3.00 to 4.00%. Distinctly, Methionine occurred in low amounts (2.19±0.01 to 2.28±0.01%). The non-essential amino acids did not significantly respond to gamma irradiation. Glytamic acid (14.32±0.01 to 14.98±0.01%) dominated this group of amino acids, distantly followed by Aspatic acid (6.22±0.01 to 6.31±0.01%). The concentrations of Threonine, Serine, Glycine and Alanine ranged from about 3.00 to 4.00% while Proline and Tyrosine were present in low amounts (<3.00).

Table 3: Essential amino acid composition (g/100 protein) of gamma irradiated Cirina forda

Parameters (%)	Gamma Irradiation dose (Gy)			
	0	10	20	50
Lysine	4.12±0.006a	4.14±0.01a	4.18±0.01a	4.09±0.01a
Histidine	3.01±0.01a	3.09±0.01a	3.04±0.01a	3.11±0.006a
Arginine	6.86±0.007a	6.72±0.01a	6.55±0.01a	6.78±0.01a
Cystine	3.11±0.01b	2.25±0.01ab	2.19±0.01a	2.24±0.01a
Valine	3.12±0.01a	3.16±0.01a	3.13±0.01a	3.22±0.01a
Methionine	2.21±0.01b	2.19±0.01a	2.25±0.01a	2.28±0.01a
Isoleucine	3.18±0.01a	3.26±0.006a	3.24±0.006a	3.16±0.01a
Leucine	7.08±0.01a	7.1±0.06a	7.11±0.01a	7.20±0.01a
Phenylalanine	5.05±0.01a	5.41±0.01a	5.16±0.01a	5.26±0.006a

Values are mean of three determinations ± SEM

Table 3: Non-Essential amino acid composition (g/100 protein) of gamma irradiated Cirina forda

Parameters (%)	Gamma Irradiation dose (Gy)			
	0	10	20	50
Aspartic acid	6.29±0.007a	6.24±0.01a	6.31±0.01a	6.22±0.01a
Threonine	4.30±0.01a	4.27±0.01a	4.28±0.01a	4.24±0.01a
Serine	3.44±0.01a	3.46±0.01a	3.41±0.01a	3.88±0.01a
Glutamic acid	14.98±0.01a	14.58±0.01a	14.32±0.01a	14.35±0.01a
Proline	2.57±0.01a	2.48±0.006a	2.32±0.01a	2.41±0.01a
Glycine	3.10±0.01a	3.14±0.01a	3.08±0.01a	3.09±0.006a
Alanine	3.69±0.01a	3.72±0.01a	3.63±0.01a	3.65±0.01a
Tyrosine	2.68±0.01a	2.78±0.01a	2.69±0.01a	2.60±0.01a

Values are mean of three determinations ± SEM

Discussion

The development of *C.forda* at molecular level, as indicated by the results of proximate analysis, showed significant response to gamma irradiation; and such effects were dose-dependent. While, the amounts of crude protein, crude fibre and ash accumulated by the *C.forda* larvae depreciated by exposure to gamma irradiation, those of dry matter and carbohydrate responded positively to the treatment. The ether extract and organic matter components of the eggs.

The significant responses of critical proximate components of *C.forda* to gamma irradiation treatment indicate that this strategy may be used in manipulating nutritional values derivable from the insect species for meeting human needs, especially in areas where *C.forda* is a stable food item. According to Shantibala, et al. (2014), insects generally offer an important nutritional resource for humans and are worthy of development in various bio-prospecting aspects including molecular approach. The inverse relationship between crude protein and intensity of gamma irradiation explains the results obtained by Odeyemi, et al. (2013) who, while studying the influence of gamma irradiation on larval productivity of *C.forda*, reported significant inhibitory effects of increasing gamma ray doses on larval development generally. Protein accumulation is a critical requirement for new tissue formation, a precursor for anatomical differentiation and metamorphosis in insects interestingly [14]. And of great value to entomophagy, the results of this study (e.g; the Control experiment) showed that *C.forda* is relatively rich in protein, as the mean 54.82% obtained here-in compares favourably with the protein values reported for a foremost edible insect source of protein, i.e., Zonocerus variegatus grasshoppers (50.39% - 53.10%) and even the highly priced periwinkle, Pachy-melania bryonensis with 55.00% protein constituent [15]. This finding thus, suggests that *C.forda* could contribute sustainably to the recommended human daily protein requirement put at 23% - 50% by the National Research Council (1980).

The carbohydrate content of 16.30% obtained from the *C.forda* insects in the Control experiment (i.e insects not exposed to gamma irradiation) was considerably higher than those reported for other popular edible insects namely cricket (5.1%) and large grasshoppers (2.2%) [16]. Also, the amounts of carbohydrate in *C.forda* increased significantly to 21.50% on exposure of the eggs to gamma irradiation at a dose of 50Gy.

This result shows that gamma irradiation can be used to significantly improve the carbohydrate constituent of *C.forda*, thus enhancing its status not only as a rich source of protein but carbohydrate also. This will no doubt, change the popular belief among entomologists and nutritionists that insects are not a good source of carbohydrate for meeting human needs, as according to Alamu, et al. (2013), the average human adult requires about 400g to 500g carbohydrate intake as starch.

The crude fibre content of 3.93% recorded for the control insects in this study is low, though it compares favourably with the 2.0% – 3.0% reported for other edible insects (Honeybee, Yam beetle and Palm weevil) in Nigeria [17]. The poor fibre content may be attributed to the thin flexible cuticle and hence little amount of chitin that characterizes insect larvae generally. Also the fibre content of the *C.forda* insects depreciated with exposure to gamma irradiation. This finding is an indication that this strategy may not be viable in fibre-enrichment of the insect species, despite the important roles of weight control, fat reduction and good bowel movements played by fibre in human diet, as eluded by Ekop (2004).

The mineral composition of the *C.forda* insects was significantly dominated by Mg, K, and Fe; and accumulation was equally significantly influenced by gamma irradiation. These minerals play crucial roles in proper metabolic and physiologic functioning of living systems. For example, while Mg prevents muscle degeneration, poor growth, immunologic dysfunction, etc. Iron strengthens the immune system as an anti-oxidant co-factor [18]. Therefore, *C.forda* can be consumed as a rich source of necessary nutritive mineral elements for proper functioning of the human body.

Generally, gamma irradiation had no significant effect on the amino-acid constituents of the insect species. This result further demonstrates the selective influence of gamma irradiation on the macro- and micro-nutrient contents of *C.forda* and thus, should guide the use of this strategy in improving the nutritional values of the insect species.

Conclusion

In this study, Macro and micro nutrients of Cirina forda eggs have been found to show significant response to irradiation when administered at desirable dosages [19-24].

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