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Full Length Research Article

Response of Mulberry Silkworm (*Bombyx mori* L.) (Race: $AC_1 \times AJ_1$) to Mulberry Leaf Fortified with Bambara Groundnut (*Vigna subterranean (*L) Verdc.) Flour

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ABSTRACT
Mulberry silkworm is a monophagous insect that derives all its nutritional needs from mulberry leaves. Thus, nutrition plays an important role in the growth and development of silkworm which ultimately impacts the economic traits. The present study evaluated the effect of less utilized specie in *Vigna* genera (*Vigna subterranea*) on rearing performance and cocoon traits of mulberry silkworm (Race: $AC_1 \times AJ_1$). Aqueous solution of *V. subterranean* seed powder supplemented leaves at 2.5%, 5.0%, 7.5%, and 10% were fed to the fifth instar larvae while the control was treated with just water. The experiment was laid out in a completely randomized design in four replicates. The result showed that *V. subterranean* supplemented leaves led to better larval growth and consequently reflected in improved cocoon traits as there is a significant length increase in the treated group than the control (2.50±0.2). The cocoon (1.29±0.05) and pupal (1.01±0.04) weight observed for 10% treatment was significantly higher than what was obtained in the other treatments and the control group (1.13±0.07; 0.87±0.06). Thus, it can be inferred that *Vigna subterranean* at 10% concentration can be used in mulberry leaf supplementation in sericulture.
KEYWORDS: Mulberry, Silkworm, Bambara groundnut, Sericulture

INTRODUCTION

Mulberry Silkworm (*Bombyx mori*) is a monophagous feeder that feeds exclusively on mulberry leaves from which they derive the necessary nutrients needed for their optimal growth and development, as well as the production of silk (Zhishen, 1999). They convert the plant protein derived from the mulberry leaves (Serin) to silk in the form of cocoon at the pupal stage of their life cycle (Alebiosu, 2011). Growth and development of the silkworm larvae and the quality of cocoon produced have been reported to be highly influenced by the quality of mulberry leaves (Alebiosu *et al.,* 2014). Thus, mulberry leaves have been supplemented with various nutrients as silkworms require specific amino acids, proteins, sugar and vitamins for optimum growth, improved silk quality, quantity and disease resistance.

Legumes are good sources of essential amino acids and fats. Bambara nut (*Vigna subterranea*), the third most common major legume after groundnut and cowpea on the African continent (Khan *et al*., 2021), is an indigenous grain legume grown in Africa though quite under-utilized, it has advantages over more favoured legumes in terms of nutritional value and tolerance to adverse environmental conditions. Mazahib *et al.* (2013) reported Bambara groundnut as a 'complete food' owing to its high protein, carbohydrate and fat content. Vurayai *et al.*(2011) also reported that the seed contains iron 4.9-48 mg/100 g, compared to a range of 2.0-10.0 mg/100 g for most food legumes, protein 18.0-24.0% with high lysine and methionine contents, ash 3.0-5.0%, fat 5.0-7.0%, fibre 5.0-12.0%, potassium 1144-1935 mg/100 g, sodium 2.9-12.0 mg/100 g, calcium 95.8-99 mg/100 g, carbohydrate 51-70%, oil 6-12% and energy 367-414 kcal/100 mg. Bamshaiye *et al.,* (2011) in their study also validated the earlier studies that Bambara groundnuts have been found to be richer than peanuts in essential amino acids hence its potential in food supplementation. Several studies have been conducted in an attempt to supplement mulberry leaves to provide the much needed nutrients for silk production in the form of feed flours, vitamins, and antibiotics among others. A report on the supplementation with *Vigna unguiculata* (Vitthalrao and Atharv, 2016) showed improved cocoon traits.

Mulberry leaves provide essential nutrients required for silkworm development, but their limited nutritional profile can be improved by incorporating supplemental feed ingredients, Bambara groundnut flour is known to contain high protein content and a balanced amino acid profile, making it an excellent candidate for improving the nutritional value of the silkworm diet. Investigating the response of silkworm to a diet supplemented with Bambara groundnut flour will provide valuable insights for optimizing the nutritional requirements of silkworm. The findings of the study will also serve as a foundation for future research, policy recommendations, and practical applications in the sericulture industry. Hence, this study attempts to determine the larval performance and cocoon traits of the mulberry silkworm in response to Bambara groundnut flour-supplemented mulberry leaf.

MATERIALS AND METHODS:

Study Site

This study was carried out in the Sericulture Laboratory of the Department of Forest Conservation and Protection, Forestry Research Institute of Nigeria (7.394^{o}N, 3.871^{o}E), Jericho hills, Ibadan using the standard rearing techniques (Krishnaswami, 1980) at 25±2^{0}C and 75±5% Relative

humidity. The Mulberry leaves were sourced from FRIN mulberry plantation. Before the commencement of rearing, the silkworm rearing room and equipment used for rearing were properly washed with detergent and disinfected with 3% formalin. Healthy and disease-free mulberry silkworm eggs (Race: $AC_1 \times AJ_1$), were procured from the Ondo state agri-business empowerment center (OSAEC) and incubated to hatch. After hatching, the larvae were brushed into rearing trays and fed three times daily at 8 am, 12noon, and 4 pm (Ayandokun *et al*., 2018) with fresh succulent mulberry leaves cut into 1cm by 1cm fragments to be easily consumed by the newly hatched larvae. The larvae were fed at the rate of 30g/dfl (disease-free laying), 90 g/dfl, 500 g/dfl,1000 g/dfl, and 8000 g/dfl for the 1st, 2nd, 3rd, 4th, and 5th instar larval stages, respectively as recommended by Badejo *et al*. (2009).

Preparation of Treatments and Mode of Application

Seeds of *V. subterranean* were purchased from the local market and powdered using a grinder. Finely powdered *V. subterranean* seeds were dissolved in distilled water and diluted at 2.5%, 5.0%, 7.5%, and 10% respectively while the control was treated with distilled water. Freshly harvested mulberry leaves were impregnated with V. *subterranean* seed powder by dipping in different concentrations of the seed powder solutions for 30 minutes after which the leaves were air-dried in the laboratory before feeding to the insects. The feeding of the silkworm larvae with the treated leaves commenced at the 4th larval stage and continued till the 5th larval instar stage when the insects began to pupate. Prior to the commencement of feeding the insects with Bambara groundnut mulberry-treated leaves, the first to third larval stages were fed with untreated mulberry leaves. At the 4th and 5th larval stages, the silkworms were divided into experimental groups of 50 larvae per rearing tray and arranged in a Completely Randomized Design in four replicates and fed with the treated leaves (Saranya *et al*., 2019) as the larvae feed more and longer at this stage. Before pupation, ten larvae were picked at random from the 5th larval instar in each of the treatments for weight and length measurements. The length and weight of the larvae were measured using a metler weighing balance and a graduated ruler. The larvae were transferred from the rearing trays to the mountage for cocoon spinning. The cocoons were harvested on the fifth day after mounting out of which ten (10) were randomly selected and their weights were measured. The cocoons were opened with the aid of a sharp razor blade to separate the pupa and shell. The weights of both pupae and shell were taken and recorded.

Statistical Analysis

Data collected were subjected to Analysis of Variance using SAS statistical software and means were separated using Least Significant Difference at 5% level of probability.

RESULTS

Effect of Bambara Groundnut Supplemented Mulberry Leaves on Silkworm Larvae

The result of Bambara groundnut-supplemented mulberry leaves on the silkworm larvae showed no significant difference in the weight gain of the larvae across all treatments (Table 1). The larval lengths at the concentration of 5 – 10% Bambara groundnut-supplemented mulberry leaf were not significantly different, but significantly higher than the larval lengths recorded at the control treatment and 2.5% concentration. The lengths of silkworm larvae fed with 2.5% Bambara groundnut-supplemented mulberry leaf were not significantly different from the control treatment.

Effect of Bambara Groundnut Supplemented Mulberry Leaves on Cocoon Traits

The cocoon and pupal weights recorded at a concentration of 10% of Bambara groundnut-supplemented mulberry leaves were significantly higher than in other treatments (Table 2). However, there was no significant difference in the cocoon and pupal weights at 2.5%, 5.0%, 7.5%, and the control. The shell weight was not significantly different among all the treatments

Table 1: Effect of Bambara groundnut supplemented mulberry leaf on silkworm larval weight and length

TREATMENTS	LARVAL WEIGHT (G)			LARVAL LENGTH(CM)		
	Initial	Final	Weight gain	Initial	Final	Length increase
2.5%	0.49±0.09	2.75±0.08	2.26±0.17a	2.90±0.16	5.30±0.12	2.40±0.28b
5.0%	0.32±0.03	2.85±0.03	2.53±0.00a	2.38±0.03	5.38±0.09	3.00±0.74a
7.5%	0.42±0.10	2.65±0.01	2.22±0.10a	2.77±0.23	5.33±0.06	2.56±0.17a
10%	0.61±0.04	2.98±0.14	2.37±0.18a	2.91±0.01	5.44±0.16	2.53±0.15a
Control	0.57±0.08	2.91±0.12	2.34±0.20a	2.89±0.19	5.39±0.05	2.50±0.20b

Each value is the mean of ten larvae

Numbers followed with the same letters in the column are not significantly different from one another ($p<0.05$)

Table 2: Effect of Bambara groundnut supplemented mulberry leaf on cocoon traits of silkworm

TREATMENTS	COCOON WEIGHT (G)	PUPAL WEIGHT (G)	SHELL WEIGHT (G)
2.5%	1.14±0.06b	0.90±0.05b	0.24±0.02a
5.0%	1.12±0.03b	0.87±0.02b	0.24±0.00a
7.5%	1.13±0.04b	0.90±0.03b	0.25±0.02a
10%	1.29±0.05a	1.01±0.04a	0.28±0.00a
Control	1.13±0.07b	0.87±0.06b	0.26±0.01a

Each value is the mean of ten larvae

Numbers followed with the same letters in the column are not significantly different from one another ($p<0.05$)

DISCUSSION

The Bambara groundnut (*Vigna subterranean*) seed powder supplemented mulberry leaves on the fifth instar larvae of mulberry silkworm (*Bombyx mori*) was found to show significant improvement on the economic parameters of silkworm namely the larval weight, cocoon weight, pupal weight, and shell weight. Among the treatments used, 10% Bambara supplementation showed the highest effectiveness, this is consistent with the studies of Vitthalrao and Atharv (2016) on the utilization of mulberry leaves supplemented with cowpea (*Vigna unguiculata*). The authors recorded a significant improvement in the economic parameters of the silkworm at 10% concentration among the various concentrations of the aqueous solution of cowpea. Similarly, Chairman *et al*. (2011) reported enhanced cocoon weight and other traits in silkworm reared on the black gram and groundnut seed powder-supplemented mulberry leaves. These improvements may be due to the increased protein content in the supplemented mulberry leaves. This is in agreement with the report of Eman (2020), on the increase in total protein content and economic traits of silkworms fed with soy flour and spirulina-fortified mulberry leaves.

Although mulberry leaves are complete diets for mulberry silkworms, there are possibilities of deficiencies due to variations in mulberry plants and their management. Thus, the supplementation of the leaves has been studied by many scholars to result in higher yields because the quantity and quality of silk produced by mulberry silkworm is highly dependent on larval nutrition and wellbeing. Several compounds have been used as additives in the supplementation of mulberry leave over the years; these include the use of vitamins like ascorbic acid (Talebi *et al*., 2002; Etebari *et al*., 2004), folic acid and multivitamins (Etebari *et al*., 2002), minerals (Etebari and Fazilati 2003; Jayappa *et al*., 2020), serifeed (Ananda Kumar and Micheal, 2011), botanical plant extracts (Waktole and Bhaskar, 2015), probiotics (Masthan *et al*., 2017), legumes (Saravanan Manjula *et al*., 2010, 2011; Anil Kumar and Prashanth, 2018) among others.

CONCLUSION

The study demonstrated the inclusion of a 10% concentration of Bambara groundnut-fortified mulberry leaf had a significant positive impact on the growth and development of silkworm larvae. The supplementation resulted in improved larval weight and length, indicating enhanced overall growth. Furthermore, the feeding of silkworm with Bambara groundnut supplemented mulberry leaf had a beneficial effect on the later stages of the silkworm's life cycle. The cocoon weight, shell weight and pupal weight showed significant improvements, suggesting that the supplementation will positively influence the silk production and quality.

These findings therefore highlighted the potential of incorporating Bambara groundnut as a supplement to mulberry leaf in silkworm-rearing practices to enhance the yield and quality of silk. The results of the study have important implications for silk producers and researchers involved in sericulture, providing a valuable approach to optimize silkworm growth and silk production. Further research may be warranted to explore the optimal concentration of the supplementation and its potential effects on other aspects of silkworm development and silk quality.

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