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Assessment of Three Varieties of Morus indica L. on the Growth and Performance of a Bivoltine Breed of Bombyx mori L. in Nigeria

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Abstract
The effect of three varieties of Mulberry leaves (Morus indica L.: K2, S-36 and EFG) on growth and performance of a bivoltine breed of Silkworm (Bombyx mori L.) was evaluated in a Tropical rainforest belt of South-West Nigeria. At the end of the fifth instar, S-36 variety of Mulberry leaves significantly increased the larvae mean weight to 1.28g when compared with K2 (1.07grams) and EFG (0.93grams) ($P<0.5$). Though the three varieties did not show any significant difference ($P<0.05$) in terms of weight of wet cocoons (K2 -1.83g, S-36 -1.95g and EFG -1.80g) , however S-36 significantly increased the filament length of cocoon spun by Bombyx mori when compared with others (S-36 produced mean length of 824m, K2, 594m and EFG, 432m).The results revealed that the nutritive value of Mulberry leaves varied from each other. Except for the adult Bombyx mori all other results presented a significantly higher performance of the S-36 variety above K2 and EFG variety ($P<0.05$). The result showed that S-36 significantly supported the growth and productivity of Bombyx mori than K2 and EFG leaves.
Keywords: Mulberry leaves, *Bombyx mori*, Instar, Cocoon.

1. Introduction

Silkworm (Bombyx mori L.) is the larva of the domesticated silkmoth, widely known to be the main commercial source of silk material for textile. In order to cope with different threats such as physical attack from animals, birds or insects and harsh environmental conditions, Silkworm cocoons as they transform into moths evolved a wide range of different structures and combinations of physical and chemical properties (Chen, et al., 2012). This had been processed into solid silk materials. Though there are different species of silkworms but only a few of these produce silk (Nagaraju, 2000; Mita, et al 2004; Xia, et al 2004).

In recent years, silk materials have received attention because of their attractive combinations of mechanical strength and toughness as well as environmental friendly conditions under which the materials are processed to solid fibers (Porter and Vollrath, 2009). Silk is called the queen of textiles due to its glittering luster, softness, elegance and durability. It is a very costly fiber, the price of a ton ranging from $45,000 - $80,000 (Borisade, 2012). In 2011, the income of Chinese farmers engaged in silkworm production was 22.4 billion Yuan and the value of the silk industry output was 203.8 billion Yuan (Jiang and Xia, 2014). Silkworm production will be a highly lucrative job for Nigeria farmers if the potential is properly harnessed. The silk is a natural fibrous substance obtained from cocoons spun by larvae of silkmoth and is preferred over all other types of fibers due to its remarkable properties like water absorbency, heat resistance, dyeing efficiency, and luster (Rahmathulla, 2012). B. mori has also become an important bioreactor for production of various recombinant proteins of bio-medical interest (Wang et al., 2005; Zhang et al., 2006; Shimomura et al., 2009; Zhang et al., 2009).

Sericulture, the rearing of silkworm for the production of raw silk originated in China between 2600 and 2700 BC (Barber, 1992; Rahmathulla, 2012) and was kept secret for over 3,000 years before it leaked to India, Korea and other nations in Asia and Europe (Ball, 2009; He, 2010). With recent campaign for establishment of local textile manufacturing industry, sericulture as an agro-industry is now gaining attention in Nigeria.

Bombyx mori larva is monophagous that feeds only on mulberry leaves almost constantly for 4 to 6 weeks. The growth and development of Bombyx mori is greatly influenced by quality of mulberry leaf (Rahmathulla, 2012) . Tanaka, et al., 1973, reported that silkworm from the same genetic stock responded variedly when fed on the leaves of different nutritional quality. Assessment of the nutritional quality of the three readily available varieties of Morus indica L. (K2, S-36 and EFG) in Nigeria as it affect the larval growth, weight and cocoon yield would be useful.

The study was therefore designed to rear silkworm of bivoltine breed on three different available mulberry leaves with a view to evaluating them in terms of performance and on the final yield of silk reeled from produced cocoons.

2. Materials and Methods

2.1 Study Area

The study was conducted at the Ekiti State Sericulture Development Project, Ado Ekiti, Nigeria from March to May, 2014 using the available mulberry plantations being utilized for feeding silkworms. An existing bivoltine breed of silkworm was used.

2.2 Treatment Processes

Egg production

The eggs for the experiment were produced in the standard Grainage unit. The egg sheets were dipped in 2% formalin solution for 5 minutes for surface dis-infection and later air-dried. This is necessary to enhance the attachment of the eggs firmly to the acid-resistant paper and also to disinfect against disease.

Grainage section is regarded as the "Heart of sericulture" due to the technical experience it requires to sustain the eggs of mulberry silkworm .It is a very critical area of sericulture.

Deflossing

Deflossing of the seed cocoons were done for a clear view of the shape and to ensure easy cutting. After deflossing, the seed cocoons were spread in a single layer on plastic trays.

Cocoon Cutting and Sex Separation

Cocoons were cut at both ends to facilitate emergence of moths. This was carefully done to avoid injury to the pupa. Separation of male and female pupae was carried out based on their distinguished features (Oommen, 2003).

Pupae Preservation and Collection of Moths and Coupling

Pupae were preserved at temperature ranging between 22-280C and 70-80% RH

Weak and defective moths, having deformed wings and other abnormalities were rejected for egg production. Male moths emerged earlier than female moths; therefore the male moths were preserved for the female to emerge and then pair. The pairs were kept in rearing tray.

De –Coupling and Oviposition

Mating was allowed for 2 to 3 hours. Afterwards, the moths were carefully de-paired. 20 female moths were allowed per egg-sheet and each moth is covered with 'celluse' so that the moth lays eggs within the restricted area. The egg sheets along with the female moths were kept inside the egg laying room at temperature of 250C, 80%RH in total darkness for 24hours. This is called the oviposition period.

Acid treatment

Acid treatment was carried out 24 hours after oviposition. It is a way of breaking diapause of silkworm eggs to make them hatch artificially. Acid of specific gravity of 1.10 was prepared at 28oC. The solution was verified with a hydrometer.

Incubation, Black-Boxing and Hatching of eggs

Incubation of egg is the process of subjecting the silkworm eggs to alternating light and dark period under a temperature of 25oC and relative humidity of 80% to enhance the development of the eggs to blue-egg stage. Black boxing was carried out to subject the eggs to total darkness for 48 hours. This was done to enhance uniform hatching of the eggs (Mita, et al 2004; Xia, et al 2004). The black boxed eggs were exposed on the 3rd day. Hatching occurred on the 12th day of the incubation.

Feeding of larvae after hatching

The experiment was layout using Complete Randomized Design (CRD). 12 rearing trays were utilized with each tray having one disease free layings (1dfl). The three treatments (K2, S-36 and EFG) were replicated 4 times.

The Larvae were fed with mulberry leaves at the rate of 5.3g, 26.6g, 83.3g,106.6g and 166.6g during 1st ,2nd, 3rd,4th and 5th instars respectively twice per day (Oommen , 2003).

Between 22 and 23 days of the larval stage, B. mori undergoes 4 moulting periods.

2.3 Data Collection

At Larva Stage

Body length, width and weight of the larvae were taken at the 3rd, 4th and 5th instars. The body length was measured with a meter rule, while divider was used to take the width and then measured over a meter rule. The weight was determined using electronic weighing machine.

At Cocoon (Pupa) Stage

After the 5th instar, worms stopped feeding and kick start the spinning to form cocoons. Mountages were used to ensure good formation of cocoons. At the 8th day from the commencement of spinning the harvesting of the cocoons was carried out. The cocoons were careful cut so as to have access to the Pupae. The weight of the wet cocoons and the weight of the pupae were determined using electronic weighing machine. The length and width of the pupae were equally measured as described in the case of larva.

At Adult Stage

At 12th day after the commencement of spinning the adult moth emerged. Wing span, body length, width and weight of the adult moth were measured.

Post Cocoon Assessment

The Shell ratio was determined. Shell ratio is the relationship between the weight of the pupa and the cocoon in terms of percentage.

Shell ratio was calculated as shown below:

$$\text{Shell ratio} = \frac{\text{weight of shell}}{\text{weight of cocoons}} X\ 100$$ (Somashekar, 2003)

Assessment of the Denier

Denier is the standard measurement of the silk filament. The cocoons were taken for reeling. This is the process of unwinding the silk filament from the cocoon. The cocoons were stifled, boiled for 6 minutes and boiled at 90oC before reeling process. Reeling of the selected cocoons were carried on a manually operated machine. 10 samples were randomly selected from each replicate.

$$\text{Denier formula} = \frac{\text{weight of filament}}{\text{lenght of filament}} X\ 9000$$ (Somashekar, 2003)

2.4 Data Analysis

Data obtained were subjected to analysis of variance (ANOVA) using SAS 2003 package for Completely Randomized Design and means separated by Duncan Multiple Range Test (DMRT) at 5% level of probability.

3.0 Results and Discussion

3.1 Results

3.1.1 Effect of K2, S-36 and EFG varieties of mulberry leaves on the length of B. mori larvae at 3rd, 4th and 5th instars, pupae and adults

Table 1 shows the mean length of the 3rd - 5th instar larvae, the pupae and adults of B. mori fed with K2, S-36 and EFG varieties of the mulberry leaves.

Table 1: The mean length (cm) of the larvae (3rd- 5th instar larvae), the pupae and adults of B. mori fed with K2, S-36 and EFG varieties of mulberry leaves.

Stage	Variety	Mean length
3rd instar	K2 S-36 EFG	1.38b 1.58a 1.33b
4th instar	K2 S-36 EFG	3.56ab 3.72b 3.35b
5th instar	K2 S-36 EFG	3.81a 3.91b 3.46c
Pupa	K2 S-36 EFG	2.69a 2.70a 2.53c
Adult	K2 S-36 EFG	2.23a 2.19a 2.09b

*Means followed by the same letter are not significantly different at 5% using Duncan Multiple Range Test (DMRT).

The result reveals that except for the adult B. mori all other results presented a significantly higher performance of the S-36 variety above K2 and EFG variety at 5% level using Duncan's multiple range test. The table showed that the mean length of K2 variety was 1.38b at the third instar and increased to 3.56ab and later increased to 3.80a at the fourth and fifth instar larvae respectively. In the case of the pupae, the mean length reduced to 2.69a and the adult further reduced to 2.23a.

The table also revealed that the mean length of S-36 variety was 1.58a at the third instar, 3.72b at the fourth instar and finally increased to 3.81a at the fifth instar. The pupae reduced in length to 2.70a and further reduced to 2.19a at the adult stage. Also on the table is the mean length of EFG mulberry variety which was 1.33b at the third instar and increased to 3.35b at the fourth instar. There is decrease in length at pupa and adult stage, 2.53c and 2.09b respectively.

3.1.2 Effect of K2, S-36 and EFG varieties of mulberry leaves on the width of B. mori larvae at 3rd, 4th and 5th instars, pupae and adults.

The result of the mean width of the larvae (3rd – 5th instar), the pupae and adult of B. mori fed with three varieties of mulberry leaves is presented in Table 2. The result also showed consistently higher values for S-36 above K2 and EFG varieties.

There is however no significant difference among the measurements obtained for 3rd – 4th instar larvae, however there is significant difference among the three varieties at the fifth instar. In the same trend, there is no significant difference at the pupa and adult stages of silkworm for the three varieties of mulberry leaves at 5% level using Duncan's multiple range test. On the table, the mean width K2 variety was 0.28a at the third instar and later increased to 0.44b at the fourth instar and 0.62b at the fifth instar. At the pupa stage, the mean width reduced to 0.87a while the mean width increased to 0.93a at the adult stage.

The Table also showed the mean width of the S- 36 mulberry plant which was 0.30a then increased to 0.004a and 0.65a at the fourth and fifth instar larvae respectively. There is decrease in width 0.88a and 0.93a of the pupae and adults respectively. The table also consist the mean width of B. mori at third instar fed with EFG mulberry leaves. At the third instar is 0.28a, and them increased to the 0.42b at the fourth instar and then later increased to 0.55c at the fifth instar. During the pupa state, the width increased to 0.85a and decreased to 0.83a at the adult stage.

Table 2: The mean width (cm) of the larvae (3rd- 5th instar larvae), the pupae and adults of B. mori fed with three varieties of mulberry leaves.

Stage	Variety	Mean width
3rd instar	K2 S-36 EFG	0.28a 0.30a 0.28a
4th instar	K2 S-36 EFG	0.44a 0.44a 0.42a
5th instar	K2 S-36 EFG	0.62b 0.65a 0.55c
Pupae	K2 S-36 EFG	0.87a 0.88a 0.85a
Adult	K2 S-36 EFG	0.93a 0.93a 0.83a

*Means followed by the same letter are not significantly different at 5% using Duncan Multiple Range Test (DMRT).

3.1.3 Effect of K2, S-36 and EFG varieties of mulberry leaves on the weight of B. mori larvae at 3rd, 4th and 5th, pupae and adults

Table 3 shows mean weight of the larvae (3rd – 5th instar), the pupae and adult of B. mori fed with S-36 above K2 and EFG mulberry leaves.

The result reveals that except for the pupae of B. mori all the other results presented a significantly higher performance of the S-36 variety above K2 and EFG variety at 5% level using Duncan's multiple range test.

The table showed that the mean weight of K2 variety was 0.04a at the third instar which increased to 0.56b at the fourth instar and later increased to 1.07b at the fifth instar. At the pupa stage, it increased to 1.34a.

In the case of the silkworms fed with S-36 mulberry variety, the mean weight was 0.06ab at the third instar and increased to 0.71b at the fourth instar and finally increased to 1.28b at the fifth instar during the larval stage. It was also observed that the EFG mulberry variety was 0.04a at the third instar, then increased to 0.56a at the fourth instar and later increased to 0.93a at the fifth instar of the larvae. It increased in weight to 1.04b at the pupa stage .There is significant difference at the pupa stage among the three varieties.

Table 3: The mean weight (g) of the larvae of B. mori from the 3rd – 5th instar, pupae and adults fed with three varieties of mulberry leaves.

Stage	Variety	Mean weight
3rd instar	K2 S-36 EFG	0.04a 0.06ab 0.04a
4th instar	K2 S-36 EFG	0.56b 0.71c 0.56a
5th instar	K2 S-36 EFG	1.07a 1.28b 0.93a
Pupae	K2 S-36 EFG	1.34a 1.29a 1.04b
Adult	K2 S-36 EFG	1.74c 1.79a 1.44b

*Means followed by the same letter are not significantly different at 5% using Duncan Multiple (Range Test DMRT).

3.1.4 Effect of K2, S-36 and EFG varieties of mulberry leaves on the wing span of B. mori (Moth)

Table 4 shows the mean wing span of the adult B. mori fed with K2, S-36 and EFG varieties of the mulberry leaves. There is no significant difference among the wing spans of K2, S-36 and EFG. S-36 was 1.79a and later decreased to 1.63a when fed with EFG mulberry leaves.

Table 4: The mean wing span (cm) of adult B. mori fed with K2, S-36 and EFG varieties of mulberry leaves.

Variety	Mean wing span
K2	1.79a
S-36	1.79a
EFG	1.63a

*Means followed by the same letter are not significantly different at 5% using Duncan Multiple (Range Test DMRT).

3.1.5 Effect of K2, S-36 and EFG varieties of mulberry leaves on the weight of B. mori cocoons and shell

Table 5 showed that the mean weight of wet cocoons and shell of cocoon spun by B. mori fed with the three different varieties of mulberry leaves. Though S-36 has the highest cocoon weight and shell weight when compared with others, there is no significant difference among the three varieties.

Table 5: The mean weight (g) of wet cocoons and shell weight (g) spun by B. mori fed with K2, S-36, and EFG varieties of mulberry leaves

Variety	Mean Cocoon weight	Mean shell weight
K2	1.83a	0.39a
S-36	1.95a	0.47a
EFG	1.80a	0.37a

*Means followed by the same letter are not significantly different at 5% using Duncan Multiple (Range Test DMRT).

3.1.6 Effect of K2, S-36 and EFG varieties of mulberry on cocoon shell ratio of B. mori

The result of the mean shell ratio of the cocoon of B. mori fed with three varieties of mulberry leaves (K2, S-36 and EFG) is presented on Table 6. The result showed consistently higher values for S-36 mulberry leaves above K2 and EFG varieties. When fed with K2 it was 21.00a and then increased to 22.20a with S-36 mulberry leaves and reduced to 21.00a with EFG mulberry leaves. It also indicated no significant difference among the varieties.

Table 6: The mean shell ratio (%) of the cocoon spun by B. mori fed with K2, S-36 and EFG varieties of mulberry of leaves.

Variety	Mean shell ratio
K2	21.31a
S-36	24.10a
EFG	20.55a

*Means followed by the same letter are not significantly different at 5% using Duncan Multiple Range Test (DMRT).

3.1.7 Effect of K2, S-36 and EFG varieties of mulberry leaves on the filament length of silk spun by B. mori

Presented on table 7 is the filament length of the silk spun by B. mori fed with K2, S-36 and EFG mulberry leaves when reeled on manual reeling machine. The result shows that S-36 has consistently higher values than K2 and EFG mulberry leaves.

When fed with K2 it was 594.16b and later increased to 824.69a with S-36 and reduced to 432.69b for the B. mori fed on EFG mulberry leaves. There is however a significant difference between S-36 and the other two varieties.

Table 7: The mean filament length (m) of the silk spun by B. mori fed with K2, S-36 and EFG mulberry variety. .

Variety	Mean filament length
K2	594.00b
S-36	824.81a
EFG	432.69b

*Means followed by the same letter are not significantly different at 5% using Duncan Multiple Range Test (DMRT).

Table 8: The mean filament length (m), mean filament weight (g) and mean denier of the silk spun by B. mori fed with K2, S-36 and EFG mulberry variety. .

Variety	Mean filament length	Mean filament weight	Mean denier
K2	594.00b	0.26b	3.93a
S-36	824.81a	0.32a	3.49b
EFG	432.69b	0.25b	5.20c

*Means followed by the same letter are not significantly different at 5% using Duncan Multiple Range Test (DMRT).

Note: Denier is the standard measurement of silk yarn thickness

3.2 Discussion

The results obtained show that S-36 mulberry leaves significantly affects the growth of B. mori i.e. silkworm at the larva stage. This is because the silkworm fed with S-36 plant leaves grew more in terms of length, width and weight compared to other mulberry leaves. In the same trend, at the pupa stage, it was also discovered that B. mori fed with S-36 mulberry leave grew more in length, width and weight in comparison with those fed with K2 and EFG mulberry leaves.

During the cocoon stage, B. mori fed with S-36 mulberry leaves produced the highest quality cocoon in terms of the size and weight of the cocoon (i.e. the cocoons are bigger in size compared to those that are fed with K2 and EFG mulberry leaves) and the amount of silk, reeled from the cocoons. Equally, at the adult stage, B. mori fed with S-36 mulberry leaves performed better in terms of length, width and weight. When the cocoons were reeled on using manual reeling machine, the silkworms fed with S-36 mulberry leaves produced silk fiber with the longest filament length and significantly different from others.

The result obtained which indicated highest performance exhibited by silkworms fed with S-36 was also supported by the study carried out by Ogunyemi and Popoola, 2012. S-36 Mulberry variety was equally recommended for Silkworm rearing because of its high percentage of total protein by Venkalesh and Seema, 2011. According to them, out of about seven varieties tested on B. mori, S-36 showed the highest total protein of 0.317mg/gm. This was also supported by Adeduntan and Oyerinde, 2010.

Although both K2 and S-36 perform almost the same way, the slight difference between K2 and S-36 mulberry leave is likely due to differences in protein content. But there is no difference in terms of their texture (Kawakami, 2003).

EFG mulberry leaves that exhibited least growth and cocoon yield has been reported by Davies, 2000, to have low moisture content and less nutrients. These are the major determinant factors affecting larva growth and productivity of silkworm (Borisade, 2012).

4.0 Conclusion

The results obtained from the study have pointed to the effectiveness of K2 and S-36 varieties as sources of good leaves for feeding silkworms. It is however suggested that for a successful young and late age rearing of silkworms, S-36 should be used. Apart from the nutritive value, the plant also performed well on the field. It spreads on the field and possesses the ability to suppress weeds when fully established. The leaves are large and smooth, with good rooting ability. Sericulture program should be vigorously encouraged by both State and Federal governments of Nigeria as an avenue for employment creation and poverty alleviation.

It can also successfully serve as a veritable source of foreign exchange earnings. The two most populous countries in the world (China and India) engaged their rural sectors in the practice to reduce unemployment rate and check rural - urban migration. Cottage industries can be set up to engage the youth and women. In India, spent pupae are being used as organic fertilizer and as a constituent of poultry and fish feed. This potential can equally be tapped by Nigeria farmers. In addition, B. mori has also become an important bioreactor for production of various recombinant proteins of bio- medical interest, which can serve as another way for the utilization of this economic insect in Nigeria.

Being a new area in Nigerian agricultural sector, farmers have to be supported in terms of training and resources. Mulberry is a hardy crop that has been striving well in South Western part of the Country.

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