© 2018 IJSRST | Volume 4 | Issue 2 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Treating the leaves of mulberry, *Morus alba* (L) with aqueous solution of seed powder of cowpea, *Vigna unguiculata* (L) and feeding fifth instar larvae of silkworm, *Bombyx mori* (L) for the fortification of the cocoon and silk filament

[1]Manisha Mahendra Nalwade, [2]Kajal Appasaheb Pondkule, [3]Trupti Nandkumar Gaikwad, [4]Vitthalrao Bhimasha Khyade

[1, 2, 3]Shardabai Pawar Mahila Mahavidyalay, Tal-Baramati, Dist-Pune, Maharastra, India

[4]Sericulture Unit, Malegaon Sheti Farm, Agricultural Development Trust Baramati, Shardanagar, (Malegaon Khurd) Post Box No.- 35 ,Tal. Baramati, Dist. Pune, Maharashtra, India

ABSTRACT

The cowpea (Vigna unguiculata) seed powder was dissolved in distilled water and diluted to 2.5%, 5%, 7.5%, and 10% concentrations. Fresh mulberry leaves were dipped in each concentration of aqueous solution of cowpea seed powder for half an hour. 1000 ml solution was used for 100 grams of mulberry leaves. Treated mulberry leaves were drained off completely and then used for feeding. The mulberry leaves were fed five times per day at the rate of 100 grams per 100 larvae for each time. Untreated group of larvae were feed with untreated mulberry leaves. Water treated group of larvae were feed with water treated mulberry leaves. The experimental groups of larvae were feed with feed separately with 2.5 percent cowpea treated; 5.00 percent cowpea treated; 7.5 percent cowpea treated and 10.00 percent cowpea treated mulberry leaves. Treating the mulberry leaves with various concentrations of aqueous solutions of cowpea seed powder and feeding to the fifth instar larvae of multivoltine cross breed race of silkworm, Bombyx mori (L) was found reflected into significant improvement in the weight of cocoon (31.862 Percentages); cocoon shell weight (52.336 percentages); pupal weight (26.336 percentages) and shell ratio. There was significant improvement in the silk filament length (00.323 percentages); silk filament weight (21.317 percentages) and denier scale of silk filament. Midgut enzymes (Protease, Amylase, Trehalase, Sucrase and Urease) were found influenced through treating mulberry leaves with cowpea seed powder and feeding to the fifth instar larvae of multivoltine cross breed race of silkworm, Bombyx mori (L) (Race: PM x CSR2). The contents of cowpea seeds may be associated with improvement in the growth and development through increased pattern of protein turn over and overall metabolism.

Keywords : Bombyx Mori L., Digestive Enzymes, Midgut, Morus Alba L, Vigna Unguiculata

I. INTRODUCTION

Cowpeas are one of the most important food legume crops in the semiarid tropics covering Asia, Africa, southern Europe, and Central and South America. A drought-tolerant and warm-weather crop, cowpeas are well-adapted to the drier regions of the tropics, where other food legumes do not perform well. It also has the useful ability to fix atmospheric nitrogen through its root nodules, and it grows well in poor soils with more than 85% sand and with less than 0.2% organic matter and low levels of phosphorus (Singh, et al, 2003). In addition, it is shade tolerant, so is compatible as an intercrop with maize, millet, sorghum, sugarcane, and cotton. This makes cowpeas an important component of traditional intercropping systems, especially in the complex and elegant

subsistence farming systems of the dry savannas in sub-Saharan Africa. In these systems the haulm (dried stalks) of cowpea is a valuable by-product, used as animal feed.Research in Ghana found that selecting early generations of cowpea crops to increase yield is not an effective strategy. Francis Padi from the Savannah Agricultural Research Institute in Tamale, Ghana, writing in Crop Science, suggests other methods such as bulk breeding are more efficient in developing high-yield varieties.According to the USDA food database, the leaves of the cowpea plant have the highest percentage of calories from protein amongvegetarian foods.Vigna unguiculata is a member of the Vigna (peas or beans) genus. Unguiculata is Latin for "with a small claw", which reflects the small stalks on the flower petals. There is a large morphological diversity found within the crop, and the growth conditions and grower preferences for each variety vary from region to region.In Tamil Nadu, India, between the Tamil months of Maasi (February) and Panguni (March), a cake-like dish called kozhukattai (steamed sweet dumplings – also called Sukhiyanin Kerala) is prepared with cooked and mashed cowpeas mixed with jaggery, ghee, and other ingredients. Thatta payir in sambar and pulikkuzhambu (spicy semisolid gravy intamarind paste) is a popular dish in Tamil Nadu. In Sri Lanka, cowpeas are cooked in many different ways, one of which is with coconut milk (Perrino, et al , 1993 and Blade, 1997).In Turkey, cowpeas can be lightly boiled, covered with olive oil, salt, thyme, and garlic sauce, and eaten as an appetizer; they are cooked with garlic and tomatoes; and they can be eaten in bean salad (Sharma, 1998).Cowpeas provide a rich source of proteins and calories, as well as minerals and vitamins. A cowpea seed can consist of 25% protein and is low in anti-nutritional factors (Rangel, et al, 2003). This diet complements the mainly cereal diet in countries that grow cowpeas as a major food crop (Phillips, 2003).Most cowpeas are grown on the African continent, particularly in Nigeria and Niger which account for 66% of world cowpea production. The Sahel region also contains other major producers such as Burkina Faso, Ghana, Senegal and Mali. Niger is the main exporter of cowpeas and Nigeria the main importer. Exact figures for cowpea production are hard to come up with as it is not a major export crop. A 1997 estimate suggests that cowpeas are cultivated on 12.5 million hectares and have a worldwide production of 3 million tonnes. While they play a key role in subsistence farming and livestock fodder, the cowpea is also seen as a major cash crop by Central and West African farmers, with an estimated 200 million people consuming cowpea on a daily basis.According to the Food and Agriculture Organisation of the United Nations (FAO), as of 2012, the average cowpea yield in Western Africa was an estimated 483 kg/ha, which is still 50% below the estimated potential production yield. In some tradition cropping methods the yield can be as low as 100 kg/ha. Outside Africa, the major production areas are Asia, Central America, and South America. Brazil is the world's second-leading producer of cowpea seed, producing 600,000 tonnes annually. The amount of protein content of cowpea's leafy parts consumed annually in Africa and Asia is equivalent to 5 million tonnes of dry cowpea seeds, representing as much as 30% of the total food legume production in the lowland tropics (Langyntuo, et al, 2003).

For the larval instars of silkworm, Bombyx mori (L), nutrition is considered as a major influence on the quality of the cocoons. Better cocoon production has been found to be directly related by evolving successful rearing techniques. Successful rearing mostly depends on satisfying the nutritional demands of the silkworm, since there is a strong correlation between nutrition and the physiology of growth in silkworm. The quality of leaf has a greater influence on the amount of food ingested. The nutrition, particularly as it relates to the physiology of digestion, is the most fundamental and important challenges in the sericulture. Effective culture cannot occur unless a species can be grown quickly and economically. The

Bombyx mori L. (silkworm) is a phytophagous lepidopteran insect that is monophagous feeder onMorus alba L. (mulberry leaves). According to Kellner (1887), the silkworm digests albumin, fat, and carbohydrates except cellulose. The ability of silkworm to produce and secrete digestive enzymes is to a great extent influenced by the nutrient composition of the meal. Scientists have tried alternative food for the rearing of silkworm, but they were not cost effective. So they used some nutrients, minerals and vitamins as food supplements. Mulberry leaves have been supplemented with various nutrients for silkworm feeding to promote silk quality and quantity. Mahmood et al (2002) found that silkworm larvae, when fed on mulberry leaves treated with farm yard manure and ammonia solution significantly consumed more food, gained more larval weight and produced heavier cocoons as compared with those fed on untreated leaves. Ravikumar (1988) has emphasized that the quality and the nutritional status of mulberry has a great influence on the silkworm growth, silk yield and disease resistance. Silkworm requires specific essential sugars, amino acids, proteins and vitamins for its normal growth. Javed and Gondal (2002) have also reported that silkworm fed with nitrogen and ascorbic acid supplemented mulberry leaves showed higher growth and lower mortality. Silkworm midgut digestive enzymes have been studied in detail by various scientists (Kanekatsu, 1972;Kanekatsu, 1978; Eguchi and Iwamoto, 1976; Sumida, 1994 and Abraham, et al, 1992). Rationalization of some of these enzymes is a feature of the silkworm (Kanekatsu, et al, 1989). Midgut enzyme activity is also a developmental stage dependent(Kanekatsu, et al, 1993; Sumida, et al, 1990)and the diapause nature has relevance to enzymatic activities in the midgut of silkworm(Asakawa and Hamano, 1994). The understanding of the change in the digestive physiology when supplemented with the Cowpeas (Vigna unguiculata) may help to maximize the commercial production of silkworm.Cowpeas are one of the most important food legume crops in the semi-arid tropics covering Asia, Africa, southern Europe and Central and South America. A drought-tolerant and warm-weather crop, cowpeas are well-adapted to the drier regions of the tropics, where other food legumes do not perform well. It also has the useful ability to fix atmospheric nitrogen through its root nodules, and it grows well in poor soils with more than 85% sand and with less than 0.2% organic matter and low levels of phosphorus (Singh, 2003).In addition, it is shade tolerant, and therefore, compatible as an intercrop with maize, millet, sorghum, sugarcane, and cotton. This makes cowpea an important component of traditional intercropping systems, especially in the complex and elegant subsistence farming systems of the dry savannas in sub-Saharan Africa. Research in Ghana found that selecting early generations of cowpea crops to increase yield is not an effective strategy. Francis Padi from the Savannah Agricultural Research Institute in Tamale, Ghana, writing in Crop Science, suggests other methods such as bulk breeding are more efficient in developing high-yield varieties (Scott, 2008).According to the USDA food database, cowpeas have the highest percentage of calories from protein among vegetarian foods (Shaw, 2007).Although the effects of nitrogen, vitamin, and salts supplementation on the growth of silkworm have been investigated by many researchers, the effect of mulberry leaves enriched with Vigna unguiculata was not investigated. So, the present study was aimed to find out the effective dose of Vigna unguiculata application to mulberry leaves on pupa weight, silk length and silk weight. By using the effective dose, further analysis of the activities of the digestive enzymes were done in the midgut of fourth day of fourth instar larvae of silkworm and an ultimate aim to find out whether the change in activities of the enzymes have impact on the growth and silk production of silkworm.

II. MATERIALS AND METHODS

The disease free layings (DFL) of polyvoltine crossbreed race (PM x CSR2) of silkworm were procured from sericulture unit (Central Silk Board, Ministry of Textiles, Govt. of India, "Udyog Bhavan", New Delhi-110011). They were placed at ambient temperature of 25±2° C and relative humidity of 70%--80% in an incubator for hatching. After hatching, larvae were isolated from stock culture. Soon after the fourth moult, the fifth instar larvae were divided into the groups like: Untreated Control group; Water Treated Control group; 2.5 percent cowpea treated group; 5.0 percent cowpea treated group; 7.5 percent cowpea treated group and 10.00 percent cowpea treated group. Each group consisting of 100 larvae. The larvae were reared in card board boxes measuring 22 X 15 X 5 cm 3 covered with polythene sheet and placed in an iron stand with ant wells. The cowpea (Vigna unguiculata) seeds were procured from the local market (Baramati India) through Vijay P. Pharande. The seeds were shade dried and powdered using mortar. Finely powdered Vigna unguiculata was dissolved in distilled water and diluted to 2.5%, 5%, 7.5%, and 10% concentrations. Fresh mulberry leaves were dipped in each concentration of aqueous solution of cowpea seed powder for half an hour. 1000 ml solution was used for 100 grams of mulberry leaves. Treated mulberry leaves were drained off completely and then used for feeding. The mulberry leaves were fed five times per day at the rate of 100 grams per 100 larvae for each time. Untreated group of larvae were feed with untreated mulberry leaves. Water treated group of larvae were feed with water treated mulberry leaves. The experimental groups of larvae were feed with feed separately with 2.5 percent cowpea treated; 5.00 percent cowpea treated; 7.5 percent cowpea treated and 10.00 percent cowpea treated mulberry leaves. All the groups were maintained up to on mulberry leaves upto spinning. The mature larvae were transferred on mountage for spinning. The cocoons were harvested on fifth day after mounting for spinning. The cocoons were selected randomly and processed for assessment of their weight; shell weight; pupal weight and shell ratio. Likewise, another set of cocoons were selected randomly and processed for reeling. The parameters of silk filament assessed include silk filament length (meters); silk filament weight (grams) and denier scale.

The same protocol was repeated for midgut enzyme assays, which were carried out on the fifth day of the fifth instar larvae. On the fifth day of the fifth instar, the larval midguts were separated; flushed with ice cold saline; Malpighian tubules, fat bodies were removed and midguts were homogenized in ice-cold buffer solution. The homogenate was centrifuged at 3000 r/min and the supernatant was used as the enzyme source with appropriate dilution.

Assay of urease was done by the following procedure. 0.5 ml of enzyme solution was incubated with the assay buffer consisted of 0.1 M KH 2 PO 4 (pH 7.5) containing 120 mM urea, 5 mM EDTA, 0.1% (v/v) 2-mercaptoethanol and 0.5% (w/v) ascorbic acid for 3 h at 30 °C. After incubation, the reaction was terminated by adding 1/24 volume of 1 N H 2 SO 4 . Ammonia released from urea was assayed by Nessler's method. One unit of the enzyme was defined as the amount that hydrolyzed 1 μmol of urea per min under the assay condition. Amylase activity was evaluated by the Bernfeld (1955) method using glucose as standard. The reaction mixture contained 50 mM phosphate buffer (pH 6.5), 1% starch (freshly prepared), and appropriately diluted enzyme.

Sucrase activity was measured according to the method of Ishaaya and Swirski (1970) with glucose as standard. The incubation mixture contained 50 mM phosphate buffer (pH 6.5), 3.42 M sucrose, and appropriately diluted enzyme. Trehalase activity was determined by the Dahlman (1971) method with slight modification of pH from 5.6 to 6.0. The assay mixture contained 50 mM phosphate buffer (pH 6.0),

3.78 M trehalose, and appropriately diluted enzyme. The incubation period and temperature of incubation were 30 min and 24 ± 1 °C for amylase, and 60 min and 37 °C for sucrase and trehalase. The amount of glucose liberated was measured at 540 nm after inhibition of the reaction with dinitrosalicylic acid (DNS) reagent in the cases of amylase and sucrase and with concentrated H 2 SO 4 in the trehalase assay. The mixture was boiled over a boiling water bath for 10 min and diluted with distilled water. Activity is expressed as milligrams of glucose liberated per minute per milligram of protein in all three estimations. The protease enzyme assay was carried out with the method of Eguchi and Iwamoto (1982) with slight modification of the pH of borate buffer (pH 11.0) as outlined by Sarangi (1985) using tyrosine as standard. The reaction mixture contained 1% casein, 0.1 M borate buffer (pH 11.0), and appropriately diluted enzyme. The incubation was carried out for 30 min at 30 °C. The reaction was inhibited by adding 8.2 M trichloroacetic acid (TCA) and centrifuged. The supernatant was used with 0.5 N NaOH and Folin's reagent to measure the tyrosine liberated at 660 nm. Protein content in all assays was estimated with the Folin phenol reagent (Lowery, et al, 1951) using bovine serum albumin as standard.Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a commercially available statistics software package (SPSS® for Windows, V. 16.0, Chicago, IL, USA). Results were presented as means ± SD.P values < 0.05 were regarded as statistically significant.

III. RESULTS AND DISCUSSION

The results are summarized in tables (1, 2 and 3). Treating the mulberry leaves with various concentrations of aqueous solutions of cowpea seed powder and feeding to the fifth instar larvae of multivoltine cross breed race of silkworm, Bombyx mori (L) was found reflected into significant improvement in the weight of cocoon (31.862 Percentages); cocoon shell weight (52.336 percentages); pupal weight (26.336 percentages) and shell ratio (Table 1). Treating the mulberry leaves with various concentrations of aqueous solutions of cowpea seed powder and feeding to the fifth instar larvae of multivoltine cross breed race of silkworm, Bombyx mori (L) was found reflected into significant improvement in the silk filament length (00.323 percentages); silk filament weight (21.317 percentages) and denier scale of silk filament (Table – 2). This may be due to the increased protein content of the mulberry supplemented with Vigna unguiculata. This is in agreement with the work done by Hiware (2006) regarding the increased pupa weight, silk length, and silk weight when silkworm treated with homeopathic drug Nux Vomica. There is a significant increase in the all parameters while there is no significance between the 7.5% and 10% dose of Vigna unguiculatawith respect to pupa weight, silk length and silk weight. So, 7.5% was fixed as the effective dose.

The activities of midgut Protease; amylase; Trehalase; Sucrsae and Urease in the fifth instar larvae of multivoltine cross breed race of silkworm, Bombyx mori (L) were found influenced through feeding mulberry leaves treated with various concentrated aqueous solution of cowpea seed powder. Midgut protease activity of the control group larvae was measured 36.951(±2.714) units. There was significant (5.731 to 12.386 percent) increase in themidgut protease activity through feeding the fifth instar larvae with mulberry leaves treated with cowpea seed powder. Midgut amylase activity of the control group larvae was measured 11.873 (±0.924) units. There was most significant (8 to 23 multiples) increase in themidgut amylase activity through feeding the fifth instar larvae with mulberry leaves treated with cowpea seed powder. 15.626 (±0.849) units of trehalase activity have been enrolled by the control group larvae. There was minor changes in units of trehalase activity through feeding the fifth instar

larvae with mulberry leaves treated with cowpea seed powder.14.423 (±0.971)units of sucrase activity have been enrolled by the control group larvae. Feeding the fifth instar larvae with mulberry leaves treated with cowpea seed powder was found enhanced this midgut sucrase activity from 23.684 to 103.501 percentage.

Urease catalyzes the hydrolysis of urea to produce ammonia and carbamate; the carbamate produced is subsequently degraded by spontaneous hydrolysis to produce another ammonia and carbonic acid (Zimmer,2000). Urease activity tends to increase the pH of its environment as it produces ammonia, a basic molecule. Ureases are found in numerous bacteria, fungi, algae, plants and some invertebrates, as well as in soils, as a soil enzyme. They are nickel containing metalloenzymes of high molecular weight (Krajewska, et al, 2012).In the present attempt, feeding the fifth instar larvae with mulberry leaves treated with cowpea seed powder was found increasing midgut urease activity from 17.915 to 481.54 units. Ammonia produced from urea by the action of mulberry leaf urease is assimilated into amino acids via the glutamine synthetase/glutamate synthase pathway in the same way as plants and micro organisms (Hirayama, et al, 1982).The same has also been outlined by Rosenthal et al (1982). Larvae of the bruchid beetle Caryedes brasiliensis feeds on a neotropical legume Dioclea megacarpa. It possesses high urease activity, and is capable of utilizing urea generated from canavanine, a toxic amino acid stored in the seeds of the host plant. However, the origin of the urease activity detected in the insect has not been clarified. The beetle's urease might originate from the legume seeds rather than from the insect itself as observed in the silkworm. Generally, legume seeds have high urease activity (Rosenthal, 1974).Therefore, in the present study the Vigna unguiculata, the leguminous plant, may also have more urease activity. Urea utilization as a nitrogen source for protein synthesis has been well studied in mammals (Liu, et al, 1955; Rose and Dekker, 1956; Snyderman, etal, 1962; Grimson, et al, 1971 and Richards, 1972) and chicks (Okumura, et al, 1979). It has been shown that intestinal flora was indispensable for utilizing urea-nitrogen for protein synthesis (Levenson, et al, 1959 and Deguchi, et al, 1978). The urea recycling system found in the silkworm more or less resembles that of mammals, but it is noteworthy that silkworm utilizes an enzyme of the host plant for the insect and that urea metabolism in silkworm is completely dependent on the diets that the insect is given. Mulberry leaf urease and the supplemented Vigna unguiculata urease may make a significant contribution to silk production in the silkworm by converting useless urea into ammonia available as a nitrogen source of silk-protein. As the pupa weight, silk length and silk weight are significantly increased upon supplementation of Vigna unguiculata, it is in agreement with Hirayama (1994), who found that the silk production of the larvae reared on mulberry leaves was larger than that of the larvae reared on the artificial diet.Poor nutrition and low-nutrient diets have direct effects on primary biochemical and physiological systems, and thus may decrease the performance of insects by effecting changes in the detoxification system that can alter the susceptibility of the insect (Lindroth, et al, 1991), the poor feeding behavior may be correlated with the alteration in digestive enzyme activity on insecticide treatment (Vyjayanthi, et al, 2002). In the present study activity of the enzymes amylase, sucrase, and protease were increased, which may be due to the sufficient amount of substrate resulting from high food intake. Sumida et al (1990) have reported that midgut sucrase is activated by sucrose at a higher concentration (<100 mM) derived from the ingested food in the midgut lumen. The rational food consumption by a lepidotpteran larva was correlated directly with the activities of amylase and invertase by Christopher and Mathavan (1985) with the larva receiving 100% food found to have the highest amylase and invertase activities, which declined as the percentage of food offered was reduced. Similarly, the heavier pupa weight on Vigna unguiculata supplementation could

have resulted in the increased activity of amylase and sucrase.Digestion of leaf proteins is aided by the proteolytic enzymes, proteases. Late silkworms are generally eat coarse leafs, and are suppose to have a highly specific protease enzyme system that hydrolyzes the fibrous protein found in abundance in coarse mulberry leaves (Ito and Arai, 1966).The proteolytic activity of the alimentary canal in relation to feeding of proteins has been studied in many insects (Dad, 1956 ; Hamano and Mukaiyama, 1970). In the present study, protease activity has been increased on Vigna unguiculata supplementation and it is presumed that the bean may activate the enzyme molecules to act on their substrates, or the enzyme molecules may be have sufficient amount of substrate. Protease activity is influenced by the age, sex, and feeding behavior of silkworms and decreases significantly on starvation during late fifth instars (Jadhav and Kallur, 1988). The observations of the present investigation can thus be correlated with increased feeding behavior and increased quantity of food ingested by the silkworm for the active participation of these enzymes in the process of digestion, which in turn reflects in the high pupa weight, silk length and silk weight.Trehalase activity to the contrary, was inhibited in the midgut of silkworms supplemented with Vigna unguiculata. Azuma and Yamashita (1985) reported an increase in midgut trehalase activity serves for the utilization of hemolymph trehalose for metabolic energy to maintain active processes in various situations, such as starvation. The decreased trehalase activity may also be due to decreased hydrolysis of trehalose to release glucose molecules in drastic conditions and in high energy demand (Hasegawa and Yamashita, 1970).The energy demand, which might have been supplied in the form of glucose molecules by the hydrolysis of trehalose in the midgut by trehalase (Nath, 2000). In this regard, Singaravelu et al (2004) have also been observed that there was a significant decrease in the ovarian enzymes when the silkworms were fed with mulberry leaves sprayed with biocide azadirachtin. In the present study as the trehalase activity was significantly decreased it is presumed that there is no such disturbance in the carbohydrate metabolism and also no drastic situations for the silkworm.Conclusively enough, the supplementation of Vigna unguiculata at the level of 7.5% may have beneficial effects on the growth of the silkworm and also increase the quantity of the silk production by enhancing the digestibility of the mulberry leaves. So, this supplementation could be prescribed to the farmers to get more quantity of silk.

IV. CONCLUSION

The cowpea (Vigna unguiculata) seed powder treatment to mulberry leaves and feeding to the fifth instar larvae of multivoltine cross breed race of silkworm, Bombyx mori (L) was found reflected into significant improvement in the weight of economic parameters (cocoon; cocoon shell weight; pupal weight and cocoon shell ratio) of silkworm, Bombyx mori (L). The activities of midgut Protease; amylase; Trehalase; Sucrase and Urease in the fifth instar larvae of multivoltine cross breed race of silkworm, Bombyx mori (L) were found influenced through feeding mulberry leaves treated with various concentrated aqueous solution of cowpea seed powder. Midgut protease activity of the control group larvae was measured 36.951(±2.714) units. There was significant (5.731 to 12.386 percent) increase in themidgut protease activity through feeding the fifth instar larvae with mulberry leaves treated with cowpea seed powder. Midgut amylase activity of the control group larvae was measured 11.873 (±0.924) units. There was most significant (8 to 23 multiples) increase in themidgut amylase activity through feeding the fifth instar larvae with mulberry leaves treated with cowpea seed powder. 15.626 (±0.849) units of trehalase activity have been enrolled by the control group larvae. There was minor changes in units of trehalase activity through feeding the fifth instar larvae with mulberry leaves treated with cowpea seed

powder.14.423 (±0.971)units of sucrase activity have been enrolled by the control group larvae. Feeding the fifth instar larvae with mulberry leaves treated with cowpea seed powder was found enhanced this midgut sucrase activity from 23.684 to 103.501 percentage. Midgut urease activity was found increased from 17.915 to 481.54 units. The urease activity is the index of metabolism. Significant changes in the midgut urease activity through cowpea seed powder treatment to mulberry leaves and feeding them to larval instars of silkworm, Bombyx mori (L) in the present attempt proves fortification of the feed.

V. ACKNOWLEDGEMENT

The work had been supported by Agricultural Development Trust, Baramati Tal. Baramati; Dist. Pune - 412204 India through "Career Oriented Program in Sericulture". The research work is the part of Environmental Science Project (Curriculum of S. Y. B. Sc. Savitribai Phule Pune University) carried out by Manisha Mahendra Nalwade; Kajal Appasaheb Pondkule and Trupti Nandkumar Gaikwad for academic year: 2017 – 2018. Academic help received from Agriculture Development Trust deserve appreciation & exert salutary influence.

VI. REFERENCES

[1]. Kellner O, Kakizaki S, Matsuoka M, Yoshu T. XXIV. On the physiology of the silk worm. By Alexander pringle jameson and william ringrose gelston atkins. Landw Versuchs-stationen 1887;33:381.

[2]. Mahmood R, Jan MT, Khan MI. Effect of nitrogen (farmyard manure + urea) treated mulberry trees on the larval development and cocoon weight of silkworm, Bombyx mori L. Asian J Plant Sci 2002;2:93-4.

[3]. Ravikumar C. Western ghat as a bivoltine region prospects, challenges and strategies for its development. Indian Silk 1988;26:39-54.

[4]. Sengupta K, Singh BD, Mustafij C. Nutrition of silkworm. Bombyx mori L.I. Studies on the enrichment of mulberry leaf with various sugars, proteins, aminoacids and vitamins for vigorous growth of the worm and increased cocoon crop production. Indian J Sci 1972;11:11-27.

[5]. Javed H, Gondal MH. Effect of food supplementation by N and Ascorbic Acid on larval mortality of silkworm (Bombyx moriL.). Asian J Plant Sci 2002;1:556-7.

[6]. Kanekatsu R. Amylase in the digestive juice of silkworm larvae, Bombyx mori. J Seric Sci 1972;41:445-51.

[7]. Kanekatsu R. Studies on further properties for an alkaline amylase in the digestive juice of silkworm, Bombyx mori. J Fac Text Sci Technol 1978;76:1-21.

[8]. Eguchi M, Iwamoto A. Alkaline protease in the midgut tissue and digestive fluid of silkworm, Bombyx mori. Insect Biochem 1976;6:491-6.

[9]. Sumida M, Yuan XL, Matsubara F. Sucrase activity and its kinetic properties in pertrophic membrans, and in membrane-bound and soluble fractions of midgut in silkworm, Bombyx mori. Comp Biochem Physiol A 1994;108:255-64.

[10]. Abraham EG, Nagaraju J, Datta RK. Chemical studies of amylases in the silkworm, Bombyx mori L.: Comparative analysis in diapause and nondiapause strains. Insect Biochem Mol Biol 1992;22:867-73.

[11]. BLADE, S. F., SHETTY, S. V. R., TERAO, T. & SINGH, B. B. (1997) Recent developments in cowpea cropping systems research. IN SINGH, B.B., MOHAN RAJ, D. R., DASHIELL, K. E. & JACKAI, L. E. N. (Eds.) Advances in Cowpea Research. International Institute of Tropical Agriculture and Japan International Research Center for Agricultural Sciences.

[12]. Kanekatsu R, Ichimura H, Hori M. Distribution and developmental changes in midgut sucrase

activity of the silkworm,Bombyx mori. J Seric Sci Japan 1989;58:517-23.

[13]. Kanekatsu R, Satoh M, Kodaira R, Miyashita T. Midgut sucrase-1 (suc-1) of the silkworm, Bombyx mori: Geneties and changes in the activities during the pupal}adult development. J Seric Sci Japan 1993;62:13-9. LANGYINTUO, A. S., LOWENBERG-DEBOER, J., FAYE, M., LAMBERT, D., IBRO, G., MOUSSA, B., KERGNA, A., KUSHWAHA, S., MUSA, S. & NTOUKAM, G. (2003) Cowpea supply and demand in West and Central Africa. Field Crops Research, 82, 215-231.

[14]. Sumida M, Yuan X L, Mari YI. Mori H, Matsubara F. Changes in kinetic parameters and total activity of midgut sucrase in the silkworm, Bombyx mori during larval pupal}adult development. Comp Biochem Physiol B 1990;96:605-11.

[15]. Asakawa H, Hamano K. Enzymatic properties of digestive amylase isozymes in silkworms, Bombyx mori L. J Seric Sci Japan1994;63:13-20.

[16]. Singh B. "Improving the production and utilization of cowpea as food and fodder". Field Crops Research 2003;84:169-50.

[17]. Scott C. "Sub-Saharan Africa news in brief: 25 March-9 April". Science and Development Network. Available from:http://www.scidev.net/en/sub-suharan-africa/news/sub-saharan-africa-news-in-brief-25-march-9-april.html. [Last accessed on 2008 Apr 10].

[18]. Shaw M. "100 Most Protein Rich Vegetarian Foods". SmarterFitter Blog. Available from:http://smarterfitter.com/blog/2007/10/28/100-most-protein-rich-vegetarian-foods/. [Last accessed on 2007 Oct 28].

[19]. Singh, B.; Ajeigbe, H. A.; Tarawali, S. A.; Fernandez-Rivera, S.; Abubakar, M. (2003). "Improving the production and utilization of cowpea as food and fodder". Field Crops Research 84: 169–150. doi:10.1016/S0378-4290(03)00148-5.

[20]. Bernfeld P. Enzymes of carbohydrate metabolism: Amylases, á and â. Methods in Enzymology In: Colowick SP, Kaplan NO, editors. New York: Academic Press; 1955;1:149-58.

[21]. Ishaaya, Swirski E. Invertase and amylase activity in the armoured scales Chrysomphalus aordun and Aonidiella auranti. J Insect Physiol 1970;16:1599-606 .

[22]. Dahlman DL. Purification and properties of trehalase from tobacco hornworm larvae. J Insect Physiol 1971;17:1677-87.

[23]. Eguchi M, Iwamoto A. Comparison of three alkaline proteases from digestive fluid of the silkworm, Bombyx mori. L. Comp Biochem Physiol B 1982;71:663-8.

[24]. Sarangi SK. Alkaline protease in the midgut of the silkworm, Bombyx mori L: Changes during metamorphosis. Proc Indian Acad Sci (Anim. Sci.) 1985;94:567-72.

[25]. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.

[26]. Hiware CJ. Effect of fortification of Mulberry leaves with homeopathic drug Nux Vomica on Bombyx Mori. L. Homeopathy 2006;95:148-50.

[27]. Hirayama C, Konno K, Shinbo H. The pathway of ammonia assimilation in the silkworm Bombyx mori. J Insect Physiol 1997;43:959-64.

[28]. Rosenthal G. A. , Hughes C, Janzen DH. l-Canavanine, a dietary source for the seed predator Caryedes brasiliensis (Bruchidae). Science 1982;217:353-5.

[29]. Rosenthal GA, The interrelationship of canavanine and ureas in seeds of the Lotidae. J Exp Bot 1974;25:609-13.

[30]. Liu CH, Hays VW, Svec HJ, Catron DV, Ashton GC, Speer VC. The fate of urea in growing pigs. J Nutr 1955;78:57-72.

[31]. Rose WC, Dekker EE. Urea as a source of nitrogen for the biosynthesis of amino acids. J Biol Chem 1956;203:107-21.
[32]. SHARMA, H. C. (1998) Bionomics, host plant resistance, and management of the legume pod borer, Maruca vitrata. Crop Protection, 17, 373-386.
[33]. Snyderman SE, Holt LE, Dancis J, Roitman E, Boyer A, Balis ME. "Uuessential" nitrogen: A limiting factor in human growth. J Nutr 1962;78:57-72.
[34]. Grimson RE, Bowland JP, Milligan LP. Use of nitrogen- 15 labelled urea to study urea utilization by pigs. Can J Anim Sci 1971;51:103-10.
[35]. Richards P. Nutritional potential of nitrogen recycling in man. Am J Clin Nutr 1972;25:615-25.
[36]. Okumura J, Tanaka H, Muramatsu T. Incorporation of 15Nurea in chicks. Jpn J Poult Sci 1979;16:45-8.
[37]. Levenson SM, Crowly LV, Moriwitz RE, Malm OJ. The metabolism of carbon-labeled urea in the germfree rats. J Biol Chem 1959;234:2061-2.
[38]. PERRINO, P., LAGHETTI, G., SPAGNOLETTI ZEULI, P. L. & MONTI, L.M. (1993) Diversification of cowpea in the Mediterranean and other centres of cultivation. Genetic resources and crop evolution, 40, 121-132
[39]. PHILLIPS, R. D., MCWATTERS, K. H., CHINNAN, M. S., HUNG, Y. C., BEUCHAT, L. R., SEFA-DEDEH, S., SAKYI-DAWSON, E., NGODDY, P., NNANYELUGO, D. & ENWERE, J. (2003) Utilization of cowpeas for human food. Field Crops Res., 82, 193-213.
[40]. Deguchi E, Niiyama M, Kagota K, Namioka S. Role of intestinal flora on incorporation of 15N from dietary, 15N-urea, 15Ndiammonium citrate into tissue proteins in pigs. J Nutr 1978;108:1572-9.
[41]. Hirayama C. Effect of mulberry leaf powder addition in artificial diet on the excretion of nitrogenous products and utilization of nitrogen in the silkworm, Bombyx mori (in Japanese with English summary). J Sericult Sci Japan 1994;63:206-13.
[42]. Krajewska B, van Eldik R, Brindell M (13 August 2012). "Temperature- and pressure-dependent stopped-flow kinetic studies of jack bean urease. Implications for the catalytic mechanism". JBIC Journal of Biological Inorganic Chemistry 17 (7): 1123–1134.
[43]. Lindroth RL, Barman MA, Weisbra AV. Nutritient deficiencies and the gypsy moth, L. dispar: Effects on larval performance and detoxification enzyme activities. J Insect Physiol 1991;37:45-52.
[44]. Vyjayanthi N, Subramanyam MV. E!ect of fenvalerate- 20EC on sericigenous insects. I. Food utilization in the late-age larva of the silkworm, Bombyx mori L. Ecotoxicol Ecol 2002;53:206-11.
[45]. Christopher MS, Mathavan S. Regulation of digestive enzyme activity in the larva of Catopsilia crocale (Lepidoptera). J Insect Physiol 1985;31:217-21.
[46]. Ito T, Arai N. Amino acid requirements in Bombyx mori. J. Insect Physiol 1966;23:861-9.
[47]. Dadd RH. Proteolytic activity of the midgut in relation to feeding in the beetles, Tenebrio molitor and Ditiscus marinalis L. J Exp Biol 1956;33:311-24.
[48]. Hamano K, Mukaiyama F. Some properties of digestive fluid proteases in the silkworm, Bombyx mori, with reference to the relation between dissociation degree and nutritive value of some proteins. J Sericult Sci Japan 1970;39:371-6.
[49]. Jadhav G, Kallapur VL. Influence of age, sex and feeding on the protease activity of certain tissues of fifth instar silkworm,Bombyx mori. Entomon 1988;13:289-93.
[50]. Azuma M, Yamashita O. Cellular localization and proposed function of midgut trehalase in

silkworm larva, Bombyx mori. Tissue Cell 1985;17:539-51.

[51]. Hasegawa K, Yamashita O. Mode d'action de I'hormone de diapause dans le metabolisme glucidique de ver aand soie,Bombyx mori L. Ann Endocrinol 1970;31:631-6.

[52]. Nath BS. Changes in carbohydrate metabolism in haemolymph and fatbody of the silkworm, Bombyx mori L., exposed to organophosphorus insecticides. Pestic Biochem Physiol 2000;68:127-37. RANGEL, A., DOMONT, G. B., PEDROSA, C. & FERREIRA, S. T. (2003) Functional properties of purified vicilins from cowpea (Vigna unguiculata) and pea (Pisum sativum) and cowpea protein isolate. Journal of agricultural and food chemistry, 51, 5792-5797.

[53]. Singaravelu G, Sumathi S, Prabu P, Jagapriya L. Biological activity of azadirachtin on certain reproductive aspects of female moth of Bombyx mori L. Toxicol Int 2004;11:27-31.

[54]. Zimmer M (Apr 2000). "Molecular mechanics evaluation of the proposed mechanisms for the degradation of urea by urease". J Biomol Struct Dyn. 17 (5): 787–97.doi:10.1080/07391102.2000.10506568. PMID 10798524.

Table 1. Influence of provision of mulberry leaves treated with seed powder cowpea (Vigna unguiculata L) on the cocoon parameters of silkworm, Bombyx mori (L) (Race: PM x CSR2).

Serial No.	Parameter → Group ↓	Cocoon Weight (Grams)	Shell Weight (Grams)	Pupal Weight (Grams)	Shell Ratio%
1.	Untreated Control	2.153 (± 0.03)	0.428(± 0.019)	1.725	19.879
2.	Water Treated Control (00.00% Cowpea)	2.155 (± 0.048)	0.429 (± 0.031)	1.726	19.907
3.	2.5% Cowpea	2.264** (±0.08)	0.473** (±0.051)	1.791**	20.892**
4.	5.0% Cowpea	2.417*** (±0.11)	0.541*** (±0.053)	1.876***	22.383***
5.	7.5% Cowpea	2.786 *** (±0.19)	0.637 *** (±0.089)	2.149***	22.864***
6.	10.0% Cowpea	2.839*** (±0.23)	0.652 *** (±0.095)	2.187***	22.967***

1. Each figure is the mean of three replications.
2. Figures in parenthesis with ± sign are the standard deviations.
3. *= $P < 0.005$; ** = $P < 0.01$ And *** = $P < 0.001$

Table 2. Influence of provision of mulberry leaves treated with seed powder cowpea (Vigna unguiculata L) on the Silk Filament parameters of silkworm, Bombyx mori (L) (Race: PM x CSR2).

Serial No.	Parameter → / Group ↓	Silk Filament Length (Meters) (A)	Silk Filament Weight (Grams) (B)	Denier Scale of Silk Filament
1.	Untreated Control	796.53 (±19.271)	0.258 (±00.001)	2.915
2.	Water Treated Control (00.00% Cowpea)	801.44 (±23.582)	0.256 (±00.001)	2.890
3.	2.5% Cowpea	813.53*(±31.314)	0.277*(±00.006)	3.064*
4.	5.0% Cowpea	864.09* *(±59.953)	0.283* *(±00.008)	2.947* *
5.	7.5% Cowpea	891.38* * *(±57.816)	0.294* * *(±00.006)	2.968* * *
6.	10.0% Cowpea	799.11* * *(±62.086)	0.313* * *(±00.009)	3.143* *

1. Each figure is the mean of three replications.
2. Figures in parenthesis with ± sign are the standard deviations.
3. * = P < 0.005 ; * * = P < 0.01 And * * * = P < 0.001

Table 3. Influence of provision of mulberry leaves treated with seed powder cowpea (Vigna unguiculata L) on the activity of midgut enzymes in the fifth instar larvae of silkworm, Bombyx mori (L) (Race: PM x CSR2).

Serial No.	Midgut Enzymes → Group ↓	Protease	Amylase	Trehalase	Sucrase	Urease
1.	Untreated Control	36.951 (±1.753)	11.873 (±0.924)	15.626 (±0.849)	14.423 (±0.971)	17.915 (±0.887)
2.	Water Treated Control (00.00% Cowpea)	36.601 (±2.714)	11.861 (±1.093)	15.621 (±1.048)	14.424 (±0.969)	18.474 (±1.015)
3.	2.5% Cowpea	39.069* *(±2.887)	96.551* (±8.868)	15.783* (±1.917)	17.839* (±2.668)	136.89* *(±11.188)
4.	5.0% Cowpea	39.187* *(±2.892)	153.23* *(±26.786)	15.818* *(±2.012)	24.543* *(±3.891)	247.92* *(±18.453)
5.	7.5% Cowpea	41.423* * *(±2.219)	277.58* * *(±33.893)	13.641* * *(±1.796)	29.247* * *(±2.673)	473.93* * *(±29.627)
6.	10.0% Cowpea	41.528* * *(±3.986)	279.27* * *(±48.523)	13.572* * *(±1.876)	29.351* * *(±3.923)	481.54* * *(±33.786)

1. Each figure is the mean of three replications.
2. Figures in parenthesis with ± sign are the standard deviations.
3. * = P < 0.005 ; * * = P < 0.01 And * * * = P < 0.001