EFFECT OF ARTIFICIAL DIET COMPONENTS WITH ANTIOXIDANT ACTIVITY ON MULBERRY LEAF DEPENDENT SILKWORM REARING SYSTEM

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Abstract

Silk is an only naturally produced animal fiber. Till today, no other fabrics can match it in luster and elegance. The best-known type of silk is obtained from the cocoons of the larvae of the mulberry silkworm (*Bombyx mori* L.). Silkworm totally depends on mulberry leaves for survival. Nowadays sericulture faces problem due to labour and land crisis. To recover this problem, many scientists are trying to establish a good artificial diet based rearing system in sericulture. They use different essential food ingredients as artificial diet. On the other hand herbivores insects face oxidative stress due to allelochemical reactions with their host plant. So, to reduce oxidative stress we in this study, have tried to prepare high antioxidant rich food for silkworm larvae. We have estimated antioxidant activity of different essential food used as ingredients in artificial diet. In our experiment we pasted cooked wheat, cornflower, soybean, rice, ***Aloe vera*** gel, potato starch, and complete artificial diet separately on the mulberry leaf surface. Antioxidant activity was measured from all extracted sample separately. On the other hand silkworm larvae have been fed with pasted leaves also. The present day study suggests that the artificial diet components contain a highly potential antioxidant property. The results indicate that soybean has maximum impact on improving cocoon shell weight when compared with control. Our results indicate that the mulberry leaves when applied with specific diet component may improve the quantity of silk as revealed from enhancement of cocoon shell weight. This knowledge may have a serious impact on sericulture industry specially silk worm rearing system providing scope for improving silk quality in a more efficient way.

Keywords : Silkworm, antioxidant, artificial diet

Introduction:

Silk, the queen of textile is the only naturally produced animal fiber. The best-known type of silk is obtained from the cocoons of the larvae of the mulberry silkworm (*Bombyx mori* L.). Mulberry leaf is the only food for Silkworm survival. The present problem of sericulture industry is the

crisis of labour and land for mulberry cultivation. Different workers are experimenting to establish a good quality artificial diet based rearing system in sericulture. They use different essential food as ingredients to make effective nutritious artificial diet. Artificial diet was applied for the first time in sericulture in Japan in 1977 for the rearing of young larvae of the silkworm in cooperative rearing houses. The practical application of artificial diet in sericulture is to save supply of mulberry leaves for the rearing and to rear young healthy larvae of silk worm. Rajaram *et al.,* (2012) [1], successfully reared silkworm larvae up to chawki level by feeding **"serinutrid"** artificial diet without hampering economical attributes of silkworm rearing. Hayashiya *et al.,* (1963)[2] reported that 20-25% mulberry leaf powder, on the basis of dry weight of artificial diet, showed better larval growth and cocoon production in comparison to artificial diet without mulberry leaf powder. Any artificial diet is prepared by using minimum few basic ingredients namely, mulberry powder, soya powder, salt, sugar, and water. [1]

In present study an effort was made to investigate antioxidant activity of different artificial diet ingredients like defatted soybean meal, wheat meal, rice meal, corn starch, potato starch and *Aloe vera* gel along with S1 mulberry leaf. The antioxidant activity of complete artificial diet was also investigated. For preparation of artificial diet we have followed the process of [1] with some modifications.

Materials and methods:

Materials:

Leaves of mulberry plant (S1 variety) at mature stage, and larvae (fifth instars) of *Bombyx mori* (F1 variety) collected from Sericulture farm of Malda district and Sericulture farm of Matigara, Siliguri, West Bengal, India. Potato starch, soybean meal, rice meal, wheat meal, corn flour meal and *Aloe vera* gel were collected from local market of Siliguri.

Methods:

Extraction procedure:

Mulberry leaves were collected and the petiole was removed and washed thoroughly to remove the dust particles and left for drying. About 2 gm of rice meal, soybean meal, wheat meal, corn flour meal were measured and boiled in distilled water to prepare uniform suspension and artificial diet was prepared by combining them in a following ratio: Dried mulberry leaf (36.0 gm), Defatted soybean meal (30.0 gm), Wheat meal (4.0 gm), Rice meal (4.0 gm), Corn starch (4.0 gm), Citric acid (4.0 gm), Ascorbic acid (2 gm), Salt mixture (3.0 gm), Agar (8.0 gm), *Aloe vera* gel (1 gm), Potato starch (4 gm) and water was added in 2.6 ml multiplication of total weight of the artificial diet component.

10 gms of leaves were measured individually each time to prepare 8 different experimental samples. Thin uniform layering on the leaf was done by each individual food component. The leaves were then kept for drying for about 4 h. After drying, the leaves were crushed in a mortar and pestle and extracted in methanol for about 2 h through the refluxing process. The extracts were collected and filtered through muslin cloths. The filtrate was then centrifuged and the supernatant was collected and subjected to evaporation in a heating mantle. Final volume make up of each sample was done with additional methanol. Total extractive values were calculated. The samples were then kept in dark bottles and stored in refrigerator for further experimental use.

Experimental insect and rearing method:

The present experiment was performed in the laboratory according to the method of Krishnaswami, et al, 1978.[3] The disease - free laying (DFL) of the silkworm breed preferred for the experiment was a F1 hybrid (Nistari × bivoltine), collected from Sericulture Farm of Malda district, West Bengal. The fifth instar larvae were utilized for the treatment. Healthy and fresh leaves of S-1 cultivars of mulberry were used in the present research work. The leaves were collected from the nearest Sericulture Farm and stored in cold to maintain its freshness.

Prior to the initiation of silkworm rearing the rearing room, plastic tray, and other materials used for rearing were sterilized. In a plastic tray, rearing of ten caterpillars was conducted by feeding with S1 mulberry cultivars as a control treatment.

Leaf treatment:

For the feeding of silkworm larvae thin uniform layering of mulberry leaf with individual artificial diet components and with complete artificial diet was done and supplied to the caterpillar. Silkworm larvae were feed about 3-4 times a day.

Rearing bed maintains procedure:

Total rearing was performed at a temperature of 28 ± 1ºc and a relative humidity of 70 ± 5 %. During feeding period only fresh S-1 leaves served in control set four times a day. The trays were placed under sufficient ventilation. Disinfection status of the room was maintained highly during rearing time. Hands were washed with disinfectant solution before handling the worms. To maintain room temperature and humidity one thermo-hygrometer was used near the larval bed. The faecal matter of larvae was continuously discarded from the tray. Dead larvae, during the rearing period were immediately removed.

Data collection:

The weight of larvae in each tray was monitored by weighing them on weighing balance daily and the growth rate pattern of caterpillar was calculated. When larvae started to spin, they were left uninterrupted for four to five days to form the cocoon. After complete cocoon formation, the weight of cocoon of each set was measured. Cocoon shell weights also were measured after the emergence of the moth from cocoon shell. Weight of male and female moth was recorded separately. Number of egg laid by per pair of moth in each set was noted. Growth index, shell ratio, Effective rearing rate (ERR), Feeding response (%) were calculated by formulae (given below). The collected data was subjected for graphical and statistical analysis (Norman and Baily, 1955).

$$\textbf{Growth index} = \frac{\text{Final weight of the larvae (g) - Initial weight of the larvae (g)}}{\text{Initial weight of the larvae (g)}}$$

$$\textbf{Shell ratio (\%)} = \frac{\text{Single shell weight (gm)}}{\text{Single cocoon weight (gm)}} \times 100$$

ERR % = $\frac{\text{Total no. of cocoons harvested}}{\text{Total no. of larvae brushed}} \times 100$

Feeding response (%) = $\frac{\text{Number of grown and healthy survival larvae}}{\text{Number of larvae brushed}} \times 100$

Weight of single cocoon = $\frac{\text{Weight of 5 male cocoons + Weight of 5female cocoons (gm)}}{\text{No. of cocoons taken (10)}}$

Single shell weight = $\frac{\text{Total shell weight of 5 male cocoon + 5 female cocoon shell (gm)}}{\text{Total no of cocoons taken (10)}}$

Study of Antioxidants:

1. DPPH free radical scavenging assay

DPPH scavenging assay was done as per method of.[4] The free radical scavenging capacity of the extracts was determined using DPPH. 2ml methanol - DPPH solution was mixed with 200µl serial dilutions of methanolic leaf extract and after 10 minutes the absorbance was read at 570 nm using spectrophotometer (Systronics, 2001). The capacity to scavenge the DPPH radical was calculated using the equation:-

DPPH scavenging effect (%) = $[(\text{Abs.}_{\text{control}} - \text{Abs.}_{\text{sample}}) / \text{Abs.}_{\text{control}}] \times 100$

Where, $\text{Abs.}_{\text{control}}$ is the initial conc. of stable DPPH radical without the test compound and Abs. sample is the absorbance of the remaining conc. of DPPH in the presence of methanol.

2. $ABTS^+$ radical cation(s) decolorization assay

The spectrophotometric analysis of $ABTS^+$ radical cation(s) scavenging activity was determined according to [5] method with some modification. This method is based on the ability of antioxidants to quench the ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm in comparison to that of BHT. The $ABTS^+$ was obtained by reacting 7 mM $ABTS^+$ radical cation(s) in H_2O with 2.4 mM Potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for overnight. Before usage, the $ABTS^+$ solution was diluted to get an absorbance of 0.750 ± 0.025 at 734 nm with distilled water. Then, 2 ml of $ABTS^+$ solution was added to 1 ml of the methanolic extract. After 10 min, percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance. Solvent blanks were run in each assay. The percentage inhibition of the samples was calculated as:

$$\text{Inhibition \%} = (1 - A/A_0) \times 100$$

Where A_0 is the absorbance at 734 nm of the control, A is the absorbance at 734 nm of the sample mixture.

3. Metal chelating activity

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of [6] with slight modification. To 0.4 ml of methanol extract, 1.6 ml of methanol was diluted and mixed with 0.04 ml of $FeCl_2$ (2 mM). After 30s, 0.8 ml Ferrozine (5 mM) was added. After 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as- Chelating rate (%) = $(A_0 - A_1) / A_0 \times 100$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

4. Superoxide anion radical scavenging activity

The superoxide radical scavenging activity was measured by the method of [7] with slight modifications. All solutions were prepared in 0.1 M phosphate buffer (pH-7.4). The reaction mixture contained 1ml of NBT solution (312 µM prepared in phosphate buffer, pH-7.4), 1ml of NADH solution (936µM prepared in phosphate buffer, pH-7.4), and 1ml of aqueous extract of different concentration. After 5mins incubation, 20µl of PMS (120 µM prepared in phosphate buffer, pH-7.4) was added to the reaction mixture. The reactant was illuminated by fluorescent lamp for 30 min. The photo-induced reactions were performed using fluorescent lamps (20W). The absorbance was measured at 560 nm against methanol as control. The inhibition percentage of superoxide anion generation was calculated by using the following formula:

$$\text{Superoxide radical scavenging effect (\%)} = [(\text{Abs.}_{\text{control}} - \text{Abs.}_{\text{sample}}) / \text{Abs.}_{\text{control}}] \times 100$$

5. Reducing power assay

The reducing power was determined by the method of [8]. 1ml extract was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (1%) and incubated at 50°C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (10%) was added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.25 ml of $FeCl_3$ (0.1%) and absorbance was measured at 700 nm. Ascorbic acid, Butylated Hydroxyanisole (BHA), Tocopherol, Trolox[9] or Butylated Hydroxytoluene (BHT) [10] can be used as positive control.

Quantitative estimation:

1. Estimation of total phenol content

Total phenol in the methanolic leaf extract of *Morus alba* was determined with Folin-Ciocalteau reagent according to the method of [11.] 1 ml of methanolic fruit extract, 1ml of 95% ethanol, 5ml of distilled water, 0.5ml 50% Folin- Ciocalteau reagent was mixed and after 5mins 5% of Sodium carbonate was added and after 1hr the absorbance value was taken at 725nm.

2. Estimation of flavonol content

Flavonol content in the methanolic leaf extract of *Morus alba* was performed by the method of Sultana *et al*., (2009) by little modification. 0.5ml of Sodium nitrite was mixed to form the reaction mixture. After 5mins 0.3ml 10% Aluminium chloride, was added and diluted with 2.5ml of distilled water and mixed well. The absorbance value was taken at 510nm.

3. Estimation of orthodihydric phenol content

The determination of Ortho-dihydric phenolic content was based on the method of Kim *et al.*, (2003)[12]. Firstly, 0.2ml of methanolic extract was diluted up to 1ml volume. Then the diluted extract was added to a mixture containing 1ml of 0.05N HCl, 1ml of Arnow's reagent, 10ml of water and 2ml of 1N NaOH. Finally, after 5 min the absorbance was recorded at 515nm. The total Ortho-dihydric phenolic content in different extracts was calculated as Catechol equivalent (CE) per gm fresh weight.

4. Estimation of total soluble sugar content

Total soluble sugar content in leaf extract of mulberry was performed by the method of Hodge *et al*., (1962)[13]. Anthrone reagent was prepared by dissolving 400mg Anthrone in 200 ml H_2SO_4. 1 ml of methanolic extract was mixed with 4 ml of Anthrone reagent. The mixture was then heated for 8 min in a boiling water bath. After boiling it was cooled rapidly and the absorbance was recorded 630 nm. The total soluble sugar content in different extract was calculated as Sucrose equivalent per gm fresh weight.

5. Estimation of total reducing sugar content

Total reducing sugar content in leaf extract of mulberry was performed by the DNSA method of Sadasivam *et al.*, 1992.[14] 1 ml of methanolic sample was mixed with 1 ml of DNSA (Dinitro salicylic acid) reagent. The mixture was then heated for 5 min in a boiling water bath. Then 1 ml of Rochelle salt solution (40%) was added to it. After boiling it was cooled rapidly under running tap water and the absorbance was recorded at 510 nm. The total reducing sugar content in different extract was calculated as Maltose equivalent per gm fresh weight.

6. Estimation of total protein content

Total protein content in methanolic leaf extract of mulberry was performed by the method of Lowry *et al.* (1951).[15] 1 ml of sample was mixed with 5 ml of alkaline copper solution and incubates at room temperature for 10 min. After incubation 0.5 ml of Folin - Ciocalteau reagent was added with the mixture and again incubates at room temperature in dark for 30 min. After incubation absorbance was recorded at 660 nm.

Results:

By performing the free radical scavenging assays and quantitative estimation of bioactive phytochemicals of the leaf extracts, it was found that the mulberry leaf have the optimum antioxidant activities and it contains a sufficient amounts of bioactive phytochemicals.

DPPH radical scavenging assay

The principle for reduction of the DPPH free radical is that the antioxidant reacts with the stable free radical DPPH and converts it to 2,2-Diphenyl-1-picryl Hydrazine. The free radical scavenging activity of mulberry leaf extracts was evaluated by means of DPPH stable radical assay and expressed as the IC_{50} (50% inhibition concentration). Results given in Figure 1(A) shows that the mulberry leaf with artificial diet exhibit the lowest IC_{50} value 7.5 mg/ml.

$ABTS^+$ radical cation(s) decolorization assay

This method is based on the ability of antioxidants to quench the ABTS radical cation, a blue/green chromophore. The free radical scavenging activity of *Morus alba* leaf was evaluated by means of $ABTS^+$ radical cation(s) decolorization assay and expressed as the IC_{50}. Results given in Figure 1(B) shows that mulberry leaf (i.e. control) exhibit the lowest IC_{50} value 90.42 mg/ml.

Metal chelating activity

This method is based on the chelating activity of the extracts for ferrous ions Fe^{2+}. The metal chelating activity of mulberry *leaf* extract was expressed as the IC_{50}. Results given in Figure 1(C) shows that the lowest IC_{50} values were exhibited by mulberry leaf in combination with potato (127.56 mg/ ml) followed by the combination of mulberry with artificial diet (127.11 mg/ml).

Reducing power assay

This method is based on the chelating activity of the extracts for ferrous ions Fe^{2+}. The reducing activity of mulberry leaf extract was expressed as mg ascorbic acid equivalent / g FWT (fresh weight tissue). Results given in Figure 1(C) demonstrated that the mulberry leaf in combination with potato exhibited the highest reducing potential and it shows the highest values (5.012 mg AAE /g FWT).

Superoxide scavenging assay

The Superoxide anions were generated using PMS / NADH system is subsequently made to reduce Nitroblue Tetrazolium which yields a chromogenic product. This is the basic principle of Superoxide assay. The reducing activity of Superoxide of mulberry leaf extract was expressed as the IC_{50}. Results given in Figure 1(E) showed that the lowest IC_{50} values were exhibited by mulberry leaf in combination with soybean (61.75 mg/ml).

Estimation of total phenol content

The total phenol content in different combination of food sources with mulberry leaf was exhibited in Figure 2(A) and was expressed as Mg Gallic acid equivalent / gm FWT(fresh weight tissue) and it was found that mulberry leaf in combination with artificial diet shows highest total protein content (5.02 mg/g) followed by mulberry (control) i.e. 4.58 mg/g .

Estimation of total flavonol content

The total flavonol content in different combination of food sources with mulberry leaf was exhibited in Figure 2(B) and was expressed as mg Quercetin equivalent / gm FWT and it was found that mulberry leaf in combination with potato shows highest total flavonol content (0.216mg/g FWT).

Estimation of total Orthodihydric phenol content

The total Orthodihydric phenol content in different combination of food sources with mulberry leaf was exhibited in Figure 2(C) and was expressed as mg Catechol equivalent / gm FWT and it was found that mulberry leaf in combination with *Aloe vera* shows highest total Orthodihydric phenol content (0.322mg/g FWT) followed by mulberry leaf in combination of potato (0.310 mg/gFWT).

Estimation of total soluble sugar content

The total soluble sugar content in different combination of food sources with mulberry leaf was exhibited in Figure 2(D) and was expressed as mg sucrose equivalent / gm FWT and it was found that mulberry leaf in combination with potato showed highest total soluble sugar content (71.56 mg/gm FWT) followed by mulberry leaf in combination of rice (63.60 mg/gm FWT).

Estimation of total reducing sugar content

The total reducing sugar content in different combination of food sources with mulberry leaf was exhibited in Figure 2(E) and was expressed as Mg Maltose equivalent / gm FWT and it was found that mulberry leaf in combination with *Aloe vera* shows highest total reducing sugar content (7.01 mg/gm FWT) followed by mulberry leaf (control) (6.32 mg/gm FWT).

Estimation of total protein content

The total protein content in different combination of food sources with mulberry leaf was exhibited in Figure 2(E) and was expressed as mg BSA equivalent / gm FWT and it was found that mulberry leaf in combination with corn flour shows highest total protein content (7.26 mg/g FWT) followed by mulberry leaf in combination with *Aloe vera* (6.93 mg/g FWT).

Results for feeding of silkworms with the mulberry leaves supplemented with different artificial diet components:

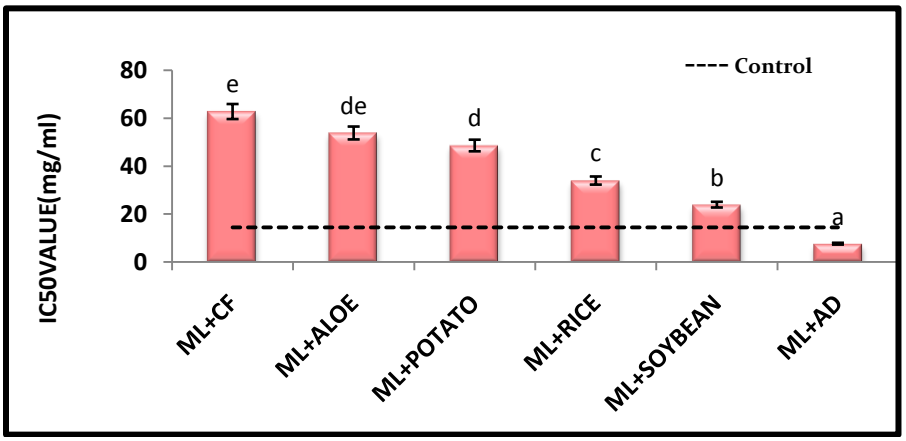

Figure 1(A) : DPPH radical scavenging activity of mulberry leaves with different components of artificial diet

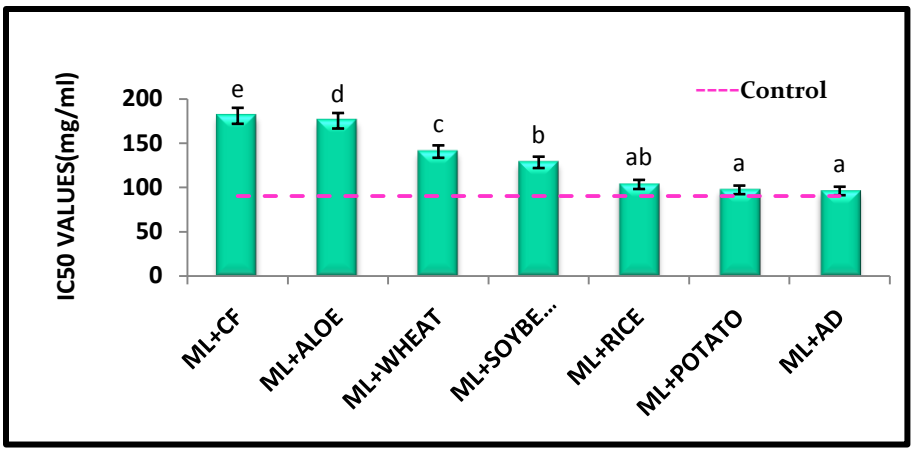

Figure 1(B) : ABTS radical scavenging activity of mulberry leaves with different components of artificial diet

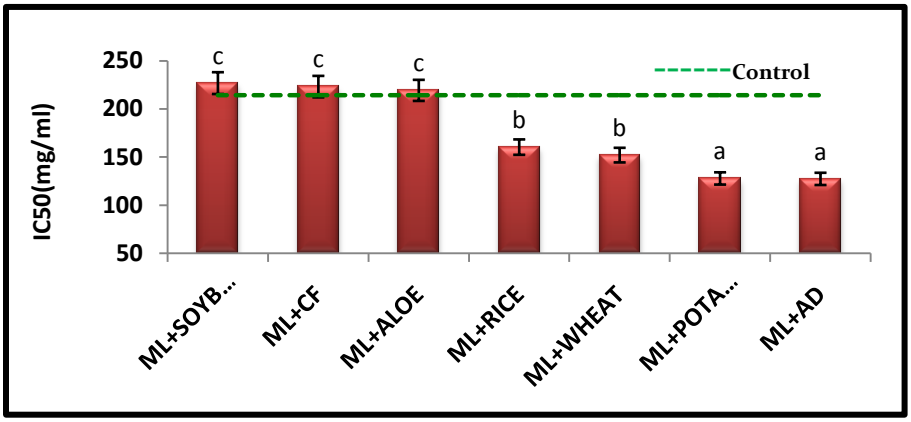

Figure 1(C) : Metal chelating activity of mulberry leaves with different components of artificial diet

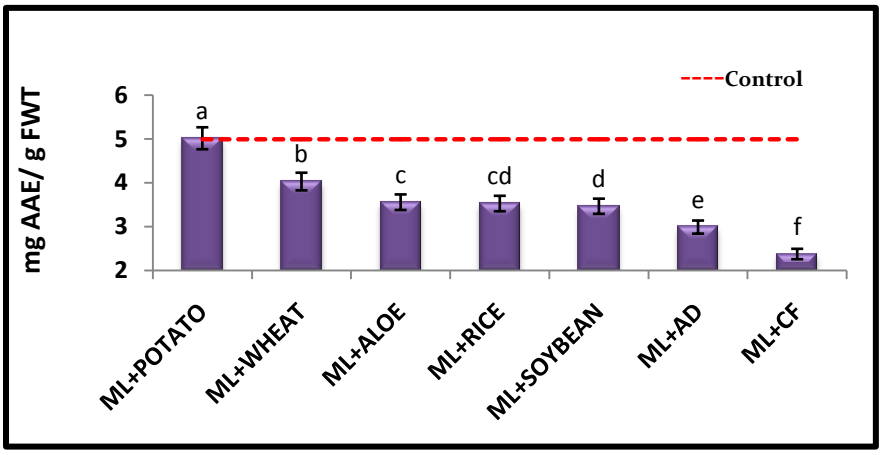

Figure 1(D) : Reducing power activity of mulberry leaves with different components of artificial diet

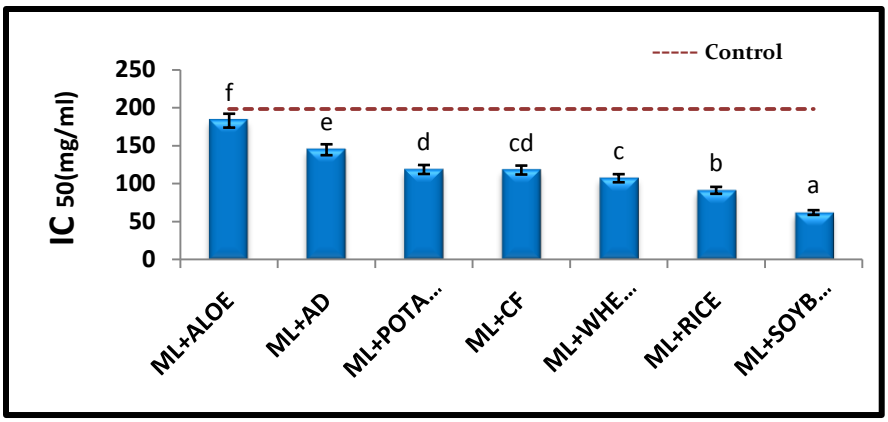

Figure 1(E) : Superoxide scavenging activity of mulberry leaves with different components of artificial diet

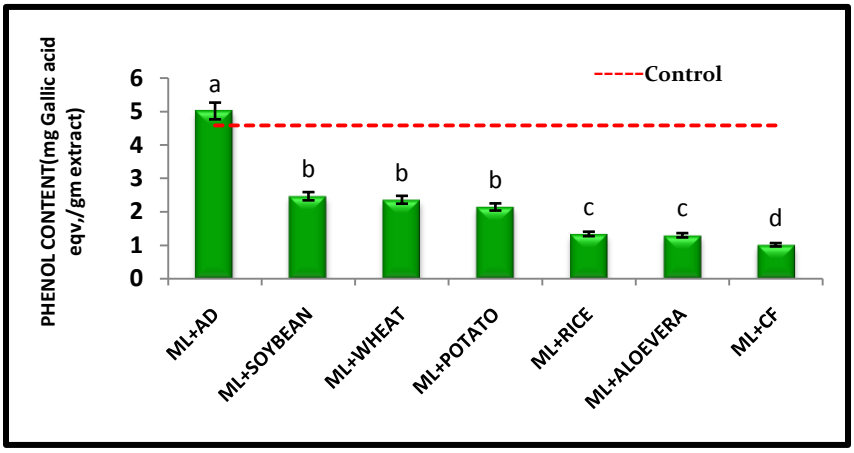

Figure 2(A) : Total Phenol Content of mulberry leaves with different components of artificial diet

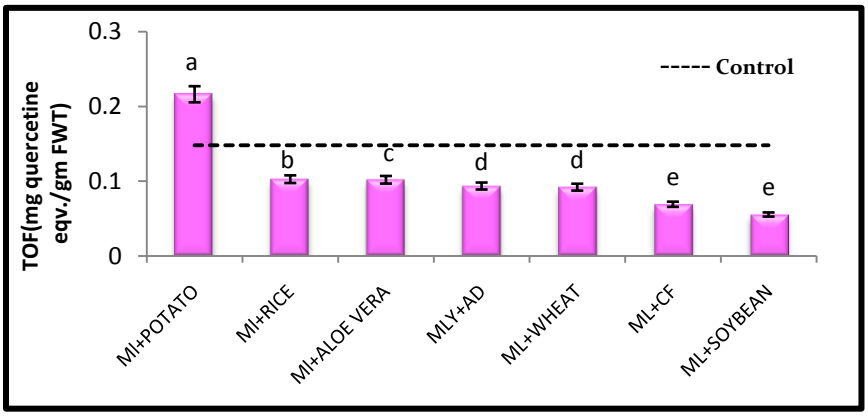

Figure 2(B) : Total flavonol content of mulberry leaves with different components of artificial diet

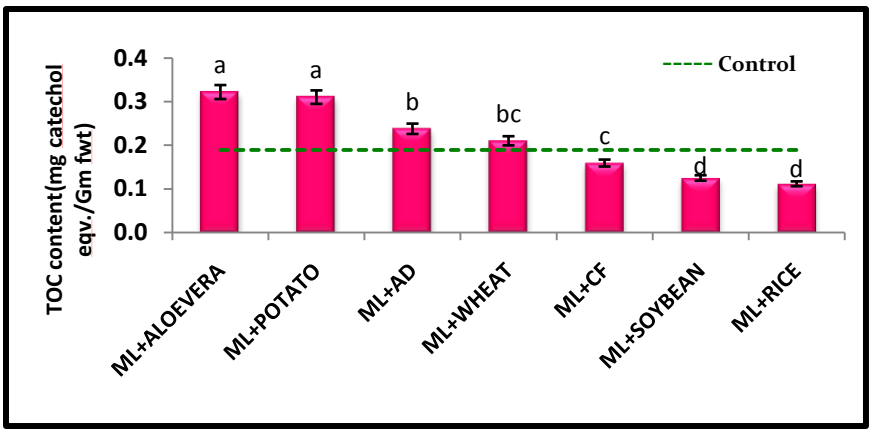

Figure 2(C) : Total orthodihydric phenol content of mulberry leaves with different food substitutions

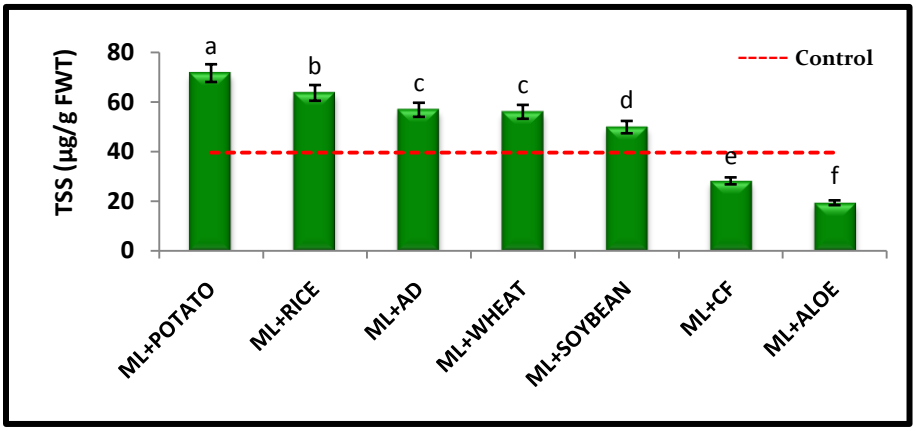

Figure 2(D) : Total soluble sugar content of mulberry leaves with different components of artificial diet

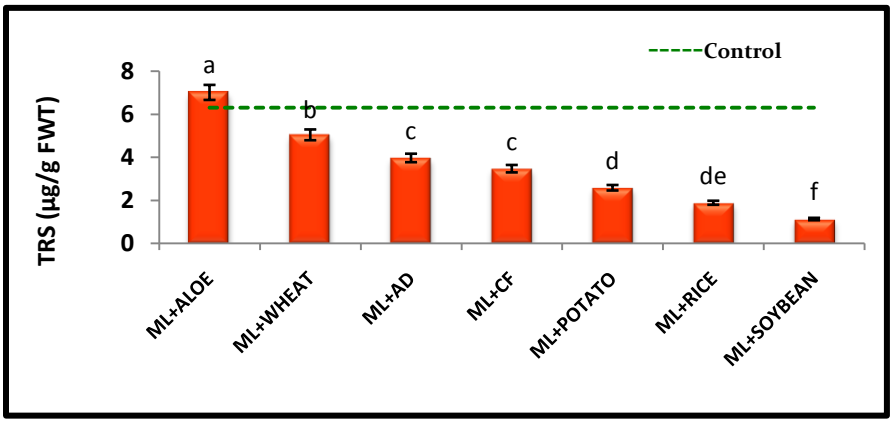

Figure 2(E) : Total reducing sugar content of mulberry leaves with different components of artificial diet

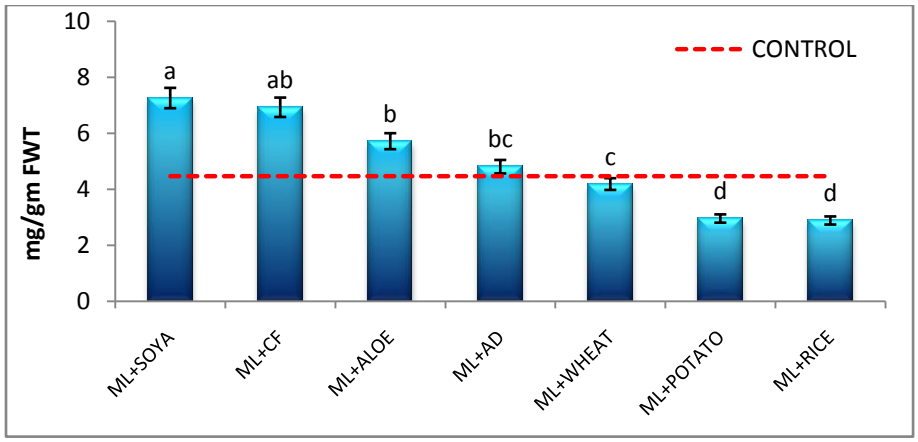

Figure 2(F) : Total protein content of mulberry leaves with different components of artificial diet

Silkworm growth curves*:*

The growth rate of caterpillar showed a parity with the control when fed with mixture of soyabean meal and mulberry leaves with days of rearing suggesting the potency of the artificial diet. It was followed by ***Aloe vera*** mixture and common artificial diet. **Fig 3(A).** The graph showed a steady increase in the growth rate by cornflour meal feeding. **Fig 3(B).** Highest cocoon weight in terms of gm was recorded by cornflour followed by soyabean and simultaneous effect was maintained by artificial diet , potato starch , *Aloe vera* in comparison to control. Minimum effect was observed by rice and wheat supplements. **Fig 3(C).** The effect of soyabean meal flour was maximum in cocoon shell weight, an increase over control. **Fig 3(D).** Both weight of the male and female moth was increased by Soyabean meal when other supplements maintained their effect over control except rice and wheat flour. **Fig 3(E), Fig 3(F).**

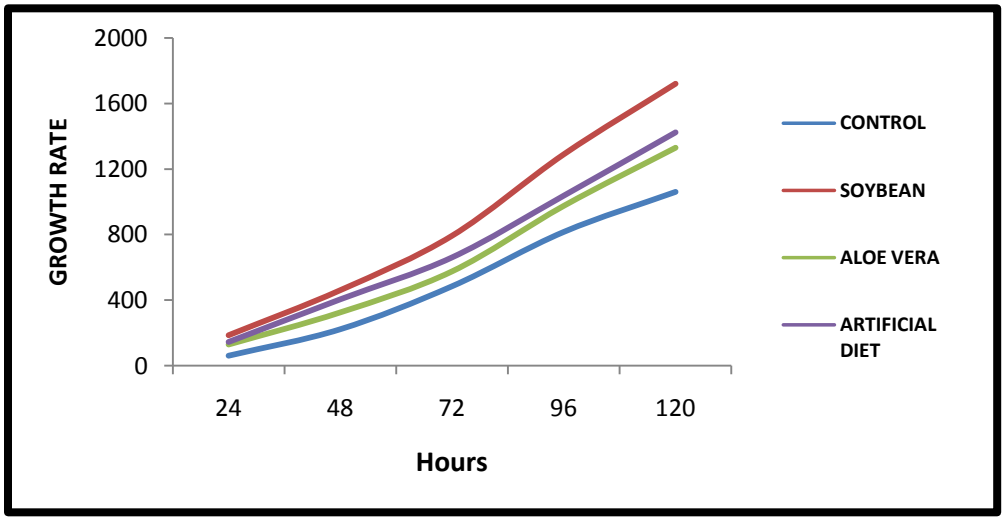

Fig 3(A): Growth rate of caterpillar after consuming other components of artificial diet except starch

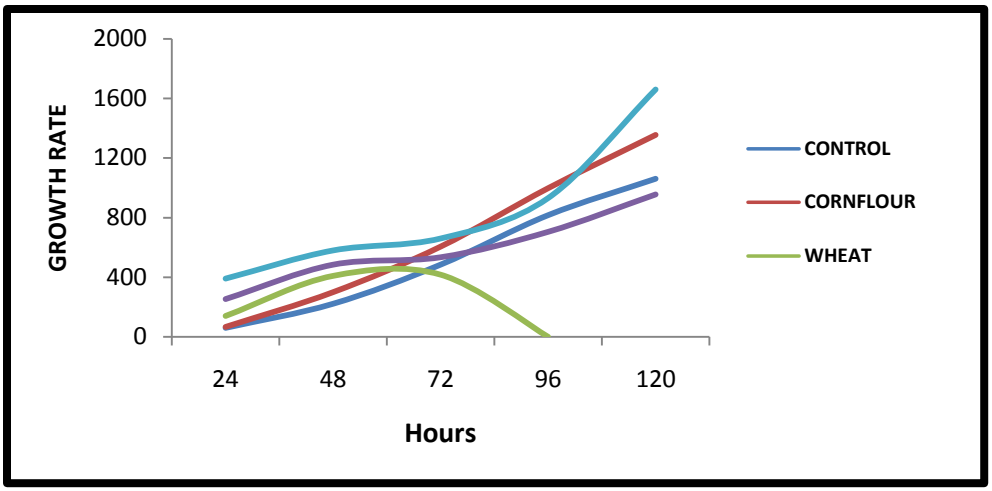

Fig 3(B): Growth rate of caterpillar after consuming starch containing food

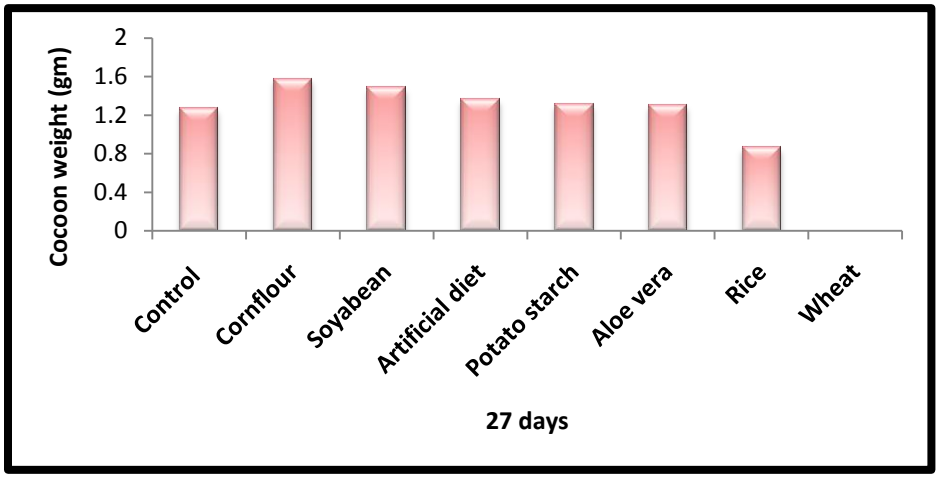

Fig 3(C): Cocoon weight after consuming different artificial diet components

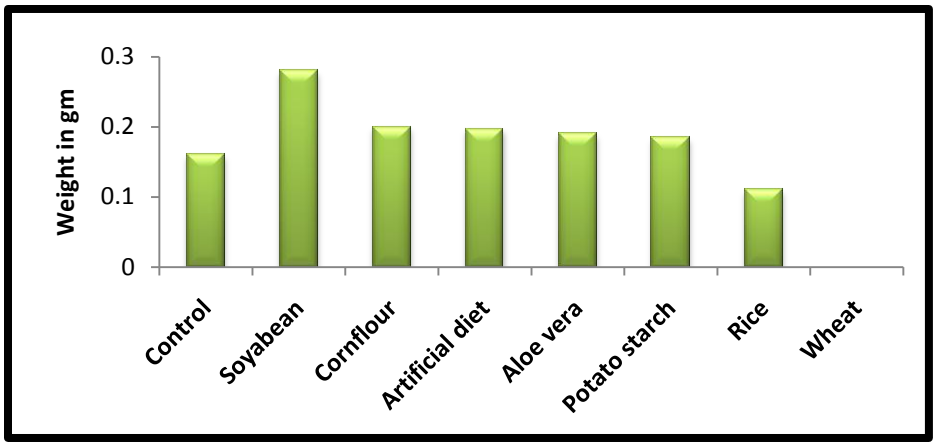

Fig 3(D): Cocoon shell weight after consuming different artificial diet components

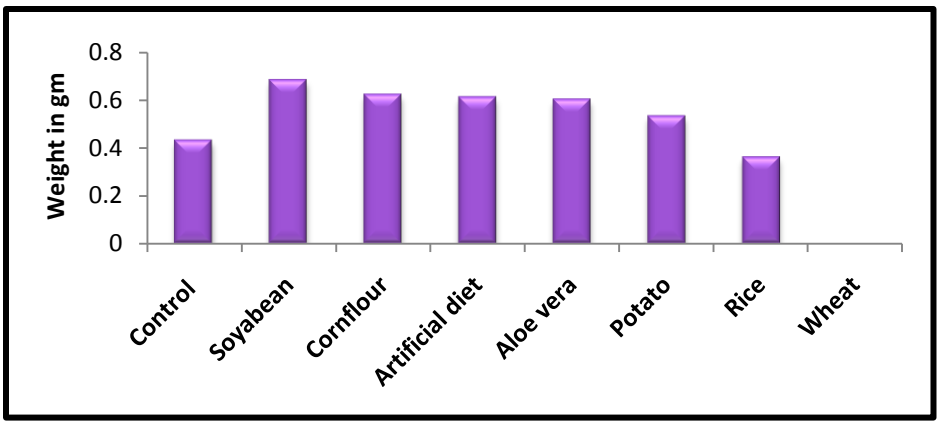

Fig 3(E): Weight of female moth after consuming different artificial diet components

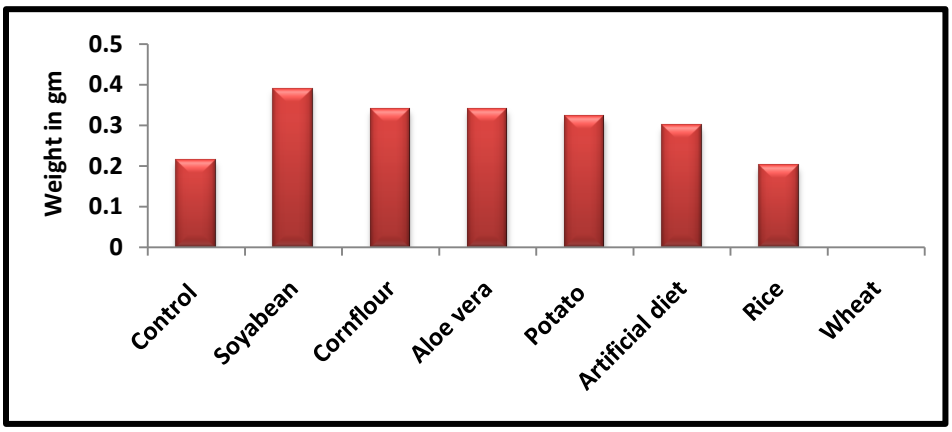

Fig 3(F): Weight of male moth after consuming different artificial diet component

Discussion:

The results presented above indicate that the different artificial diet components have different effect on the growth rate of the mature larvae and weight of cocoon, cocoon shell, male moth and female moth. It is found in the figure 3(A) and 3(B) that larvae fed on mulberry leaves supplemented with soybean meal, *Aloe vera* gel, potato starch, corn flour meal and complete artificial diet showed higher growth rate in comparison to those fed on mulberry leaf only and soybean meal had major impact on the growth rate. It was also observed that soybean meal also increases the weight of cocoon, cocoon shell, and weight of both male and female moths as found in the Figure: 3(C), (D), (E), and (F). These results are in agreement with, [16] who reported that larval growth in *Bombyx mori* was largely dependent on the dietary protein and the increase in body weight reached their maximum by diet containing soybean. The Figure 3(B) also showed that among the food sources wheat had negative impact on the growth of the silk worm larvae and after three days of treatment it led to complete mortality.

Antioxidant activity of the food components supplied with the mulberry leaves also were analyzed because the growth and development of the silkworm is sensitive to various oxidative stresses. The stable free radical DPPH has been widely used to test the free radical scavenging ability for various dietary antioxidants [17] DPPH is a free radical that remained stable at room temperature, which produces a purple colour solution when dissolved in methanol and has a absorption maxima at 517 nm. In presence of an antioxidant the purple colour imparted by DPPH fades or disappear giving rise to the colorless methanolic solution. The degree of discoloration indicates the free radical- scavenging potential of a particular antioxidant [18]. The main advantage of DPPH is that its degree of reduction by a particular antioxidant can be detected directly in a reaction through a continuous spectrophotometric analysis. Our present study demonstrates that the mulberry leaf supplemented with complete artificial diet has considerably stronger free radical scavenging potency (7.5 mg/ml) in comparison to other artificial component.

Antioxidant activity of the artificial diet used for the feeding of silkworm larvae was measured in terms of their ABTS scavenging potential. The determination of antioxidant activity is important for assessing the nutritional value of the samples [19]. In this experiment the mulberry leaf (i.e. control) showed maximum $ABTS^{+}$ radical scavenging activity [figure: 1(B)], and in case of artificial diet the complete balance diet exhibited highest activity in comparison with the individual diet components.

The chelation of transition metal ions Fe^{2+} and Cu^{2+} by artificial diet components could check the oxidation reaction.[6] The mulberry leaf supplemented with potato exhibited highest metal chelating activity followed by the combination of mulberry with artificial diet [Figure: 1(c)].

In reducing power assay, the reducing capacity of a biological compound Fe^{3+}/ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity. [20] The reducing ability of a sample was determined with ascorbic acid equivalent. Higher ascorbic acid equivalent value indicates higher reducing ability of samples. Mulberry leaf in combination with potato shows the highest reducing potential among all other artificial diet components used in the present study [Figure: 1(d)].

Super oxide is considered as an initial free radical which is formed from mitochondrial electron transport systems, to create other cell-damaging free radicals, such as hydrogen peroxide, hydroxyl radical, or singlet oxygen [21]. The result shown in Figure: 1(e) clearly indicates that mulberry leaf supplemented with soybean have the highest superoxide scavenging activity than the rest.

Phenols are very important plant product because of their scavenging capability due to presence of hydroxyl groups. The phenolic compounds may contribute directly to antioxidative damage. The result indicates that the phenol content which was found to be higher in the mulberry leaf in combination with balance diet could be partly responsible for the beneficial effects to the silkworm growth.

Flavonols are widely distributed in plants, both as co-pigments to anthocyanins in petals and also in leaves and fruits of higher plants. Like the anthocyanins, Flavonols also occur most frequently in chemical attachment with the glycosides. In many plants the participation of Flavonols in disease resistance mechanism has been widely demonstrated. The results shown in Figure: 2(B)

indicates the presence of Flavonols comparatively higher in the mulberry leaves supplemented with potato starch than other diet components.

Carbohydrates are one of the prime requirements for growth and development. Both total soluble sugar and total reducing sugar contents varies with different artificial diet components. From the Figure: 2(D) and 2(E) it can be shown that mulberry leaf supplemented with potato shows higher amount of total soluble sugar and mulberry leaf supplemented with *Aloe vera* shows higher amount of total reducing sugar content, which indicates that the respective supplementary components may serve as the exogenous source of these carbohydrates. These results are in agreement with [22] who reported that juvenile silkworm needs more carbohydrate for their growth and development.

Protein is the main component of silk and mulberry silk is mainly composed of fibroin and sericine proteins. So supply of protein containing food sources must have significant influence on the growth and development of the silkworm and the amount of silk as shown from the increase in cocoon shell weight from the Figure: 3(E) and 3(F). The Figure: 2(E) showed that the mulberry leaf supplemented with corn flour meal have higher amount of protein (7.26 mg/ml) followed by mulberry leaf supplemented with *Aloe vera* gel (6.93mg/ml).

Conclusion:

The present day study suggests that the artificial diet components contain a highly potential antioxidant activity. Considerably, high amount of bioactive phytochemicals (such as phenol, flavonol, soluble sugar, reducing sugar, protein) were estimated in these components. Results also demonstrated that the source of food rather than its intricate quality is very much important for the growth and development of silk worm larvae and for the production of silk. Prominent improvement in cocoon shell weight was observed after application of soybean, *Aloe vera*, corn flour, potato starch on mulberry leaf. The results indicate that soybean has maximum impact on improving cocoon shell weight when compared with control. Our results indicate that the mulberry leaves when applied with specific diet may improve the quantity of silk as revealed from enhancement of cocoon shell weight with additional diet components. This knowledge may have serious implication in sericulture industry applied for silk worm rearing. These results provide scope for improving silkworm rearing in more efficient way through future research.

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