



RESEARCH ARTICLE

## Modulating the Metabolic Activity of Silkworm, *Bombyx mori* L. with the Supplementation of Honey and Protein Sources

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### ABSTRACT

The present study aimed to assess the effect of honey obtained from different bee species and protein sources on the metabolic changes in mulberry silkworm, *Bombyx mori* L. The results of experiment showed that oral administration of honey and protein sources to silkworm larvae during late-age instars daily once significantly changed metabolic processes. Among the various treatments and concentrations tested, 5 per cent *Trigona iridipennis* honey significantly increased the carbohydrate in haemolymph (16.71 mg/mL) and fat body (12.38 mg/g) over all other treatments. The lipid content in haemolymph (23.03 mg/mL) and fat body (45.38 mg/g) of silkworm were also found to be superior in the larval batch treated with 5 per cent *T. iridipennis*. Apart from this, the same treatment was showed statistical superiority in increasing the activity of enzymes viz., aspartate aminotransferase (269.78 µg/mL) and alanine aminotransferase (563.25 µg/mL), which play an important role in protein metabolism.

Received : 16<sup>th</sup> July, 2021

Revised : 10<sup>th</sup> August, 2021

Revised : 25<sup>th</sup> August, 2021

Accepted : 10<sup>th</sup> September, 2021

**Keywords:** Aminotransferase; Carbohydrate; Haemolymph; Fat body; Metabolism; Honey

### INTRODUCTION

The silkworm, *Bombyx mori* L, secretes silk, a continuous and lustrous fibre having high value. The larval development and silk secreting ability of silkworm depend on the nutritional composition of food, the mulberry (*Morus* spp.) (Seidavi *et al.*, 2005; Kantwa *et al.*, 2006). Generally, the mulberry leaves contain the required amount of nutrients essential for the optimal growth and development of silkworms. However, the frequent and continuous harvesting of leaves and the inadequate quantity of fertilizers lead to the deprivation of nutrients by the silkworm. More often, this results in complete or partial reduction in cocoon and raw silk yield, ultimately huge economic loss to the farmers. This situation can best be managed by feeding the silkworm with nutritionally enriched mulberry leaves. A wide array of studies conducted on the enrichment of mulberry with various exogenous modulators having essential nutrients viz., vitamins, enzymes, hormones, minerals and amino acids have shown significant enhancement in biological as well as the cocoon characters of silkworm (Khan and Saha, 1995; Etebari *et al.*, 2004; Bhattacharya and Kaliwal, 2005; Rahmathulla *et al.*, 2007; Radjabi *et al.*, 2010; Chakrabarthy and Kaliwal, 2012; Kavitha *et al.*, 2014). One such exogenous modulator that has attracted the attention of researchers is honey

(Council of European Union, 2002). Honey contains various bioactive components in the form of free amino acids, proteins, carbohydrates, vitamins, enzymes and mineral (Falco *et al.*, 2003; Garcia *et al.*, 2005; Ball 2007; Moniruzzaman *et al.*, 2014).

The enrichment of mulberry leaves with honey and feeding to silkworm significantly enhanced the biological and economic characters of mulberry silkworm (Gad, 2013; Hassan *et al.*, 2014; Saad *et al.*, 2014; Hamzah *et al.*, 2016; Alagumanikumar and Prema, 2016; Madhavi *et al.*, 2018) and transaminases activity (Thulasi and Sivaprasad, 2015). Transaminases or aminotransferases are a group of enzymes that catalyze the interconversion of amino acids and oxoacids by transfer of amino groups (David and Zafar, 1990). Though the published information on the modulatory role of honey on silkworm economic traits is available in plenty, the comparative performance of different honey and various protein sources on the silkworm metabolism is not adequately established. Keeping in view the high nutritional and medicinal status of honey, the studies were carried out to know the impact of honey from different bee species and protein sources on the carbohydrate and lipid content in haemolymph and fat body and the activity of transaminase enzymes in mulberry silkworm.

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## **MATERIALS AND METHODS**

### **1. Silkworm culturing**

Disinfection of rearing room and equipment was performed by spraying 0.05 % Asthra solution containing high level of active chlorine at the rate of 140 mL/sq. ft. area before commencement of silkworm rearing (Sivaprasad *et al.*, 2015). The Chawki larvae of popular bivoltine Double Hybrid silkworm were procured from the Chawki Rearing Centre. Rearing was taken up by feeding nutritious mulberry leaves three times a day, under suitable ecological conditions having the temperature of  $26\pm 2$  °C and humidity of  $80\pm 5$  per cent. The mature worms were mounted using Netrika with the density of 70 larvae per sq. ft. (Dandin and Giridhar, 2014).

### **2. Preparation of test solution and application of treatment**

The required concentrations of 1, 2, 3, 4, 5 and 6 per cent of honey and protein sources were prepared by dissolving in distilled water. Weighed quantity of fresh mulberry leaves was sprayed with honey from five bee species, namely stingless bee honey, Indian bee honey, little bee honey, Italian bee honey and rock bee honey, and four protein sources such as redgram flour, horsegram flour, soya flour and pollen powder, separately. The honey of different bee species was procured from reliable sources. The raw redgram, horsegram and soyabean were roasted, ground into fine powder and sieved. The pollen pellets were collected from the bee colony using pollen trap and dried under shade.

One larval group fed on distilled water sprayed mulberry leaves and another larval group fed on mulberry leaves without any treatment (control) were maintained along with the experimental groups. The exogenous modulators sprayed leaves were shade dried for 30 minutes to remove the excess moisture and fed during third, fourth and fifth instars, once daily in the morning. The other feedings were provided with normal mulberry leaves. Each treatment was replicated thrice with 50 larvae per replication.

### **3. Biochemical analysis**

#### **i) Estimation of carbohydrate**

Three larvae per replication were taken to collect the haemolymph and fat body. Anthrone method of Seifter *et al.* (1950) was used to determine carbohydrate content in the haemolymph. One mL of the sample was added with 2 mL of 10 per cent trichloroacetic acid and 8 mL of distilled water. The collected homogenates (haemolymph and fat body) were centrifuged at 4000 rpm for 15 minutes. After centrifugation, 1 mL of the supernatant was taken and each 4 mL of freshly prepared anthrone reagent

was added. The tubes were covered with aluminium foil and kept in boiling water bath for 10 minutes. After cooling at room temperature, the colour intensity was read on the spectrophotometer at 620 nm. Glucose was used as the reference standard. The carbohydrate content of fat body was expressed as mg/g and haemolymph as mg/mL.

#### **ii) Estimation of lipids**

Three larvae per replication were taken to collect the haemolymph and fat body. The method of Folch *et al.* (1957) was used for lipid estimation, using chloroform methanol mixture (2:1). Haemolymph and fat body samples were homogenized with chloroform: methanol mixture in 1 : 10 ratio. The homogenate was then transferred to a 50 mL separating funnel and similar volume of chloroform was added. The two solvents were partitioned by the addition of 0.2 volume of water. After the funnel was shaken, the mixture was allowed to stand overnight. The lower chloroform layer containing lipid was drawn off. The lipid sample was kept in vacuum desiccators until constant weight was obtained. The lipid of fat body was expressed as mg/g and haemolymph as mg/mL.

#### **iii) Estimation of transaminase enzyme activity**

##### **a. Glutamic Oxaloacetic Transaminase (GOT) or Aspartate aminotransferase (AST)**

The colorimetric method of Reitman *et al.* (1957) was used for the estimation of glutamic oxaloacetic transaminase (Aspartate transaminase), using GOT substrate (phosphate buffer + L-aspartate + ketoglutarate). Known quantity of haemolymph was collected in each test tube and 0.5 mL GOT substrate was added. After shaking, the tubes were kept in boiling water for 60 min. Then, 0.5 mL of DNPH (dinitrophenylhydrazine) was added to the tubes and kept at room temperature. Finally, 5 mL of NaOH was added and allowed to stand for 5 min at room temperature. Then, the colour intensity was read on spectrophotometer at 503 nm.

##### **b. Glutamic Pyruvic Transaminase (GPT) or Alanine aminotransferase (ALT)**

GPT substrate (phosphate buffer + L-alanine + ketoglutarate) was used for the estimation of glutamic pyruvic transaminase (Alanine transaminase). The haemolymph was collected and GPT was quantified by adopting standard procedure (Reitman *et al.*, 1957) as described in the estimation of glutamic oxaloacetic transaminase.

#### **iv) Statistical analysis**

The data collected from the experiments were statistically analyzed in Factorial Completely Randomised Design (FCRD) as described by Panse and Sukhatme (1957).

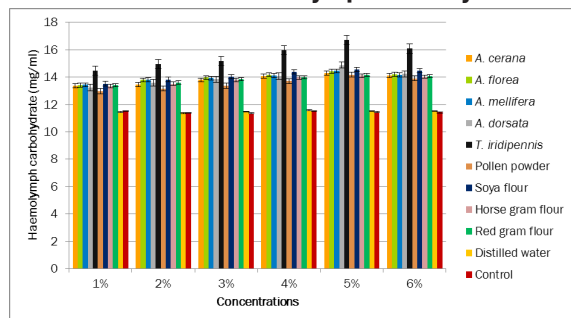
## RESULT AND DISCUSSION

The application of various honey and protein sources to silkworm larvae during the late-age stages elucidated notable changes in the metabolic activity of silkworm. This was evident from the level of variation in the carbohydrate and lipid in haemolymph and fat body, and activity of aminotransferase enzymes.

### 1. Haemolymph carbohydrate

The oral application of honey and protein sources to silkworm larvae through mulberry leaves greatly improved the level of haemolymph carbohydrate (Fig. 1). In respect of treatments, *T. iridipennis* honey significantly increased the carbohydrate content of haemolymph to 15.57 mg/mL from 11.44 mg/mL in control. This was followed by soya flour (14.13 mg/mL), *A. florea* honey (14.00 mg/mL), *A. mellifera* honey (13.99 mg/mL), *A. dorsata* honey (13.97 mg/mL), *A. cerana* honey (13.86 mg/mL) and red gram flour (13.85 mg/mL), which showed statistical parity with each other. Among the concentrations, the highest amount of haemolymph carbohydrate was recorded in 5 per cent (14.07 mg/mL), which was found to be on par with 6 (13.84 mg/mL) and 4 (13.78 mg/mL) per cent. The lowest haemolymph carbohydrate was registered in 1 per cent (13.11 mg/mL). In the interaction between concentrations and treatments, significantly more quantity of haemolymph carbohydrate was observed in 5 per cent *T. iridipennis* honey (16.71 mg/mL), which exhibited statistical parity with 6 (16.08 mg/mL) and 4 (15.98 mg/mL) per cent of *T. iridipennis* honey. The next best treatments were 3 (15.98 mg/mL) and 2 (15.17 mg/mL) per cent of *T. iridipennis* honey and 5 per cent *A. dorsata* honey (14.89 mg/mL).

**Fig 1. Impact of different honey and protein sources on haemolymph carbohydrate**



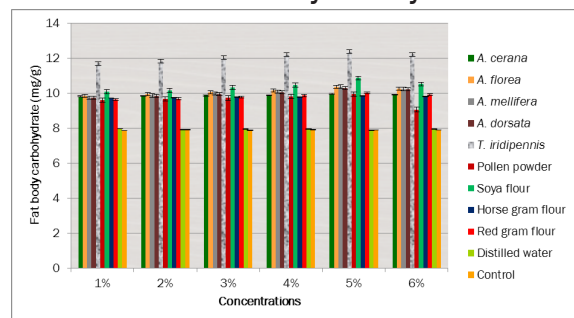
The present observations can be corroborated with the findings of Saravanan *et al.* (2021), who recorded significantly higher haemolymph glucose content ( $14.99 \pm 0.80$  mg/mL) than the control ( $13.09 \pm 0.70$  mg/mL), when the late-age silkworm was fed on mulberry leaves treated with extract of cowpea, *Vigna unguiculata* at 7.5 per cent concentration. Further, Bhattacharya and Kaliwal (2005) observed that the oral supplementation of

potassium and magnesium chloride at 150 µg/mL to fifth instar silkworm larvae once on an alternate day resulted in an increase of 44 per cent haemolymph trehalose over the untreated larvae. Kavitha *et al.* (2014) found that the oral application of zinc chloride at 100 ppm to silkworm larvae through mulberry leaves during fourth and fifth instar daily once in the evening led to significant increase in haemolymph carbohydrate level to 33.70 mg/mL from 24.0 mg/mL in control. These results conform with the present observations.

### 2. Fat body carbohydrate

Carbohydrate content of fat body considerably varied between treatments, significantly highest being in *T. iridipennis* honey (12.07 mg/g) followed by soya flour (10.41 mg/g) (Fig. 2). The treatments *A. florea* honey (10.11 mg/g), *A. mellifera* honey (10.06 mg/g) and *A. dorsata* honey (10.03 mg/g) were found to be on par with each other. Least amount of fat body carbohydrate was observed in control (7.89 mg/g). Regarding various concentrations studied, the highest level of fat body carbohydrate was registered in 5 per cent (9.99 mg/g), which showed statistical superiority over all other concentrations. This was followed by 4 per cent (9.84 mg/g), which was found to be on par with 6 per cent (9.82 mg/g). One per cent showed least amount of fat body carbohydrate (9.62 mg/g). In the interaction between treatments and concentrations, 5 per cent *T. iridipennis* honey recorded maximum quantity of fat body carbohydrate (12.38 mg/g), which was found to be on par with 6 (12.23 mg/g) and 4 (12.21 mg/g) per cent of *T. iridipennis* honey. The next better treatments were 3 (12.03 mg/g), 2 (11.83 mg/g) and 1 (11.71 mg/g) per cent of *T. iridipennis* honey.

**Fig 2. Impact of different honey and protein sources on fat body carbohydrate**



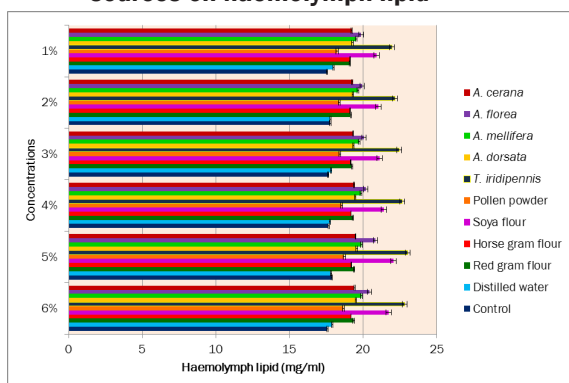
This falls in line with Bhattacharya and Kaliwal (2005) findings, who registered increased fat body glycogen, when the fifth instar silkworm was orally supplemented with potassium and magnesium chloride at 150 µg/mL. Further, the present observations are supported by Kavitha *et al.* (2014), who reported that enrichment of mulberry leaves with 100 ppm zinc chloride and feeding to silkworm larvae from the first day of fourth instar to spinning

significantly enhanced the carbohydrate in fat body (122.00 mg/g) by 82.90 per cent over the control (66.70 mg/g). Moreover, the findings of Thilsath et al. (2016) revealed that soya flour supplementation, increased the carbohydrate content of fat body compared to control also strengthens the present result of soya flour performing better, next to honey.

### 3. Haemolymph lipid

The lipid content of haemolymph showed significant variation when silkworm was reared by supplementing with honey and protein sources (Fig. 3). Among the treatments, the highest haemolymph lipid content was observed in *T. iridipennis* honey (22.52 mg/mL), which exhibited statistical superiority over all other treatments. This was followed by soya flour (21.38 mg/mL), *A. florea* honey (20.22 mg/mL), *A. mellifera* honey (19.76 mg/mL), *A. dorsata* honey (19.42 mg/mL) and *A. cerana* honey (19.35 mg/mL), which showed statistical variation among themselves. Silkworm fed with untreated leaves registered the least amount of haemolymph lipid (17.67 mg/mL). Among the different concentrations, significantly maximum haemolymph lipid content of 19.81 mg/mL was registered in 5 per cent. The next better concentrations were 6 (19.67 mg/mL), 4 (19.58 mg/mL), 3 (19.48 mg/mL) and 2 (19.42 mg/mL) per cent. The lowest haemolymph lipid was observed in 1 per cent (19.34 mg/mL). The interaction between concentrations and treatments revealed that the highest haemolymph lipid content was recorded in 5 per cent *T. iridipennis* honey (23.03 mg/mL), however, this was found to be on par with 6 (22.81 mg/mL) and 4 (22.67 mg/mL) per cent of *T. iridipennis* honey.

**Fig 3. Impact of different honey and protein sources on haemolymph lipid**



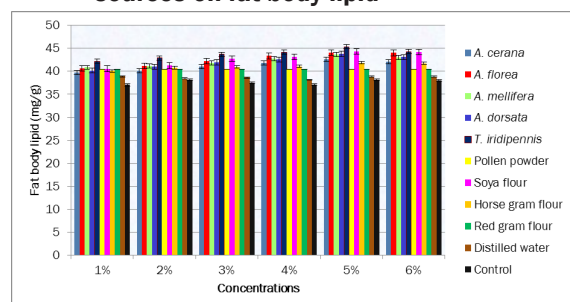
The findings of Manjula et al. (2010) showing that the *per os* administration of seed extract of Indian bean, *D. lablab* at 7.5 per cent to silkworm larvae, 5 times/day starting from third to fifth instar significantly improved the haemolymph cholesterol content to  $38.36 \pm 2.05$  from  $30.36 \pm 1.38$  mg/mL in control, falls more or less in line with the present

result. Further, the report of Saravanan et al. (2021) revealed that the application of cowpea extract (7.50 %), during late-age instar significantly increased haemolymph cholesterol, also in parity with the present result.

### 4. Fat body lipid

The present study revealed that there were significant changes in the fat body lipid content due to the application of honey and protein sources (Fig. 4). The maximum amount of fat body lipid, was recorded in *T. iridipennis* honey (43.78 mg/g), which was found to be statistically superior over all other treatments. The next better treatments were soya flour (42.71 mg/g), *A. florea* honey (42.59 mg/g), *A. mellifera* honey (42.23 mg/g), *A. dorsata* honey (42.11 mg/g), which did not show statistical variation among themselves. Control recorded the least amount of fat body lipid (37.67 mg/g). Among the concentrations evaluated, statistically greater level of fat body lipid was observed in 5 per cent (42.16 mg/g), which was followed by 6 (41.85 mg/g), 4 (41.37 mg/g), 3 (41.05 mg/g) and 2 (40.53 mg/g) per cent. Minimum amount of fat body lipid was registered in 1 per cent (40.09 mg/g). In the interaction between different treatments and concentrations, the highest fat body lipid was noticed in 5 per cent *T. iridipennis* honey (45.38 mg/g), which was found to be on par with 5 per cent soya flour (44.32 mg/g), 6 (44.31 mg/g) and 4 (44.18 mg/g) per cent *T. iridipennis* honey, and 6 per cent soya flour (44.17 mg/g).

**Fig 4. Impact of different honey and protein sources on fat body lipid**



The observation of Bhattacharya and Kaliwal (2005) showing that fortification of mulberry leaves with potassium and magnesium chloride at 50 µg/mL to fifth instar silkworm larvae once on an alternate day resulted in an increase of 25 per cent fat body lipids compared to the control, falls in line with the present finding. In addition to this, the report showing that the silkworm larvae fed on soya flour treated mulberry leaves enhanced the lipid content of fat body (Thilsath et al., 2016), substantiates the present finding of soya flour recording more fat body lipid, next to honey.

### 5. Glutamic Oxaloacetic Transaminase (GOT) or Aspartate aminotransferase (AST)

Silkworm fed on mulberry leaves sprayed with different honey and protein sources showed marked variations regarding AST activity (Table 1). Among the treatments, *T. iridipennis* honey recorded statistically greater level of AST enzyme activity of 252.31 µg/mL than all other treatments. This was followed by soya flour (249.47 µg/mL), *A. florea* honey (244.97 µg/mL), *A. mellifera* honey (242.02 µg/mL), *A. dorsata* honey (231.43 µg/mL) and *A. cerana* honey (229.58 µg/mL). The minimum activity of AST enzyme was registered in control (224.27 µg/mL). In respect of different concentrations, AST activity was noticed to be significantly more in 5 per cent (239.89 µg/mL). The next better concentrations were 6 (238.01 µg/mL), 4 (235.98 µg/mL) and 3 (233.94 µg/mL) per cent. The lowest level of AST was observed in 1 per cent (230.53 µg/mL). In the interaction between the treatments and concentrations, significantly higher AST activity was registered in 5 per cent *T. iridipennis* honey (269.78 µg/mL) than all other treatments.

This was followed by 5 per cent soya flour (264.24 µg/mL), 6 per cent *T. iridipennis* honey (260.65 µg/mL), 6 per cent soya flour (258.66 µg/mL) and 4 per cent *T. iridipennis* honey (254.36 µg/mL).

This result strengthened with the findings of Khedr *et al.* (2013) and Hassan *et al.* (2014), who reported that application of 5 per cent honey to silkworm during late-age instars significantly increased the AST activity. The supplementation of 7.5 per cent seed extract of *V. unguiculata* to silkworm from during third, fourth and fifth instars significantly enhanced the activity of AST to 420.67±22.57 IU/L from 390.21±18.22 IU/L in the untreated larvae (Saravanan *et al.*, 2021), which can also be comparable with the present results. Apart from this, the present observations on the performance of soya flour are in accordance with findings of Anil Kumar and Prashanth (2018), who registered that oral application of 6 per cent soya flour greatly increased the AST activity (278.56 µg/mL) in CSR4 silkworm breed compared to control (164.36 µg/mL).

**Table 1. Impact of different honey and protein sources on Aspartate aminotransferase (µg/mL)**

Treatments	Concentrations						Mean
	1%	2%	3%	4%	5%	6%	
<i>A. cerana</i>	227.63	228.07	229.88	230.01	231.47	230.39	229.58
<i>A. florea</i>	239.12	240.71	243.72	247.13	250.76	248.36	244.97
<i>A. mellifera</i>	236.71	238.17	240.86	242.32	248.71	245.36	242.02
<i>A. dorsata</i>	228.76	229.17	230.13	232.76	234.17	233.57	231.43
<i>T. iridipennis</i>	239.61	241.08	248.37	254.36	269.78	260.65	252.31
Pollen powder	226.29	227.06	227.94	228.27	229.31	228.96	227.97
Soya flour	237.23	239.47	245.91	251.33	264.24	258.66	249.47
Horse gram flour	225.83	226.14	227.02	228.17	229.20	228.67	227.51
Red gram flour	227.04	228.15	229.26	230.12	231.09	230.75	229.40
Distilled water	224.22	229.26	226.14	227.37	225.08	228.04	226.69
Control	223.34	224.64	224.07	223.89	224.98	224.71	224.27
Mean	230.53	231.99	233.94	235.98	239.89	238.01	235.06
	SEd			CD (0.05)			
F value	T= 1.16			T= 2.34*			
	C= 0.49			C= 1.01*			
	TC= 1.12			TC= 2.25*			

Values are mean of three replications and pooled mean to two silkworm rearing.

T - Treatment, C - Concentration, \* Significant

### 6. Glutamic Pyruvic Transaminase (GPT) or Alanine aminotransferase (ALT)

The application of honey from different bee species and protein sources resulted in elevated level of ALT enzyme activity in silkworm (Table 2). The ALT activity was significantly the highest in *T. iridipennis* honey (553.44 µg/mL), which showed statistical superiority the control (497.85 µg/ml). The next better treatments were in the order of soya flour (547.24 µg/mL), *A. florea* honey (540.85 µg/mL), *A. mellifera* honey (537.54 µg/mL) and *A. dorsata* honey (531.64 µg/mL), which exhibited statistical variation among themselves. Among the different

concentrations, 5 per cent registered the highest ALT activity of 528.94 µg/mL, followed by 6 (528.40 µg/mL), 4 (526.10 µg/mL), 3 (523.95 µg/mL) and 2 (522.36 µg/mL) per cent. The least ALT activity was registered in 1 per cent (519.36 µg/mL). The interaction between treatments and concentrations revealed that 5 per cent *T. iridipennis* honey showed maximum alanine aminotransferase activity 563.25 µg/mL, which exhibited statistical parity with 6 per cent *T. iridipennis* honey (561.18 µg/mL). The treatments, 5 per cent soya flour (558.73 µg/mL) and 4 per cent *T. iridipennis* honey (556.25 µg/mL) were found to be on par with each other.

The present observations are in conformity with results of Khedr *et al.* (2013) and Hassan *et al.* (2014), who recorded that supplementation of honey at 5 per cent on mulberry leaves significantly enhanced the ALT activity in silkworm haemolymph. Further, the findings of Manjula *et al.* (2010), who showed that the enrichment of mulberry leaves with 7.5 per cent seed extract of *D. lablab* and feeding to silkworm during late-age significantly elevated

the activity of ALT ( $440.02 \pm 23.62$  IU/L) over the untreated larvae ( $390.22 \pm 18.21$  IU/L), also fall in line with the present observations. Increased activity of ALT ( $518.01 \mu\text{g/mL}$ ) was observed, when the larvae of silkworm breed, CSR2 was fed on mulberry leaves treated with 4 per cent soya flour (Anil Kumar and Prashanth, 2018). This report can also corroborate the present result of soya flour performing well, next to honey.

**Table 2. Impact of different honey and protein sources on Alanine aminotransferase ( $\mu\text{g/mL}$ )**

Treatments	Concentrations						
	1%	2%	3%	4%	5%	6%	Mean
<i>A. cerana</i>	525.41	528.52	529.72	529.95	531.07	530.15	529.14
<i>A. florea</i>	537.53	538.12	540.01	541.25	545.81	542.37	540.85
<i>A. mellifera</i>	533.22	534.14	535.21	539.48	542.27	540.91	537.54
<i>A. dorsata</i>	524.17	526.78	527.39	531.81	540.91	538.75	531.64
<i>T. iridipennis</i>	540.29	548.37	551.28	556.25	563.25	561.18	553.44
Pollen powder	511.57	511.73	511.91	512.07	512.38	512.34	512.00
Soya flour	538.25	540.11	542.76	550.33	558.73	553.26	547.24
Horse gram flour	523.37	525.17	528.83	531.21	535.62	532.21	529.40
Red gram flour	518.15	519.08	520.28	521.81	522.19	521.63	520.52
Distilled water	482.40	479.92	492.30	486.97	484.81	490.41	486.14
Control	478.61	491.26	483.72	485.92	481.23	489.19	484.99
Mean	519.36	522.11	523.95	526.10	528.94	528.40	524.81
	SEd			CD (0.05)			
F value	T= 0.68			T= 1.37**			
	C= 0.26			C= 052**			
	TC= 2.05			TC= 4.12**			

Values are mean of three replications and pooled mean to two silkworm rearing.

T - Treatment, C - Concentration, \* Significant, \*\*Highly significant

## CONCLUSION

From the present experiments, it is crystal clear that the fortification of mulberry leaves with different honey and protein sources significantly altered the metabolic processes in *B. mori*. Among the various sources and concentrations evaluated, *T. iridipennis* honey at five per cent showed statistical superiority by registering highest carbohydrate ( $16.71 \text{ mg/mL}$  and  $12.38 \text{ mg/g}$ ) and lipid content ( $23.03 \text{ mg/mL}$  and  $45.38 \text{ mg/g}$ ) in the haemolymph and fat body, respectively over all other treatments. *T. iridipennis* honey in the same concentration was also significantly increased the activity of Aspartate aminotransferase ( $269.78 \mu\text{g/mL}$ ) and Alanine aminotransferase ( $563.25 \mu\text{g/mL}$ ) enzymes.

### Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

### Originality and plagiarism

We assure that we have written and submitted only entirely original works.

### Consent for publication

All the authors are agreed to publish this research article.

### Competing interests

There were no conflict of interest in the publication of this content

### Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail: arunaramaiah07@gmail.com

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