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Effects of methanol extract of *Pteridium aquillinum* (L) Kuhn (*Dennstaedtiaceae*), on some commercial and physiological parameters of silkworm, *Bombyx mori*

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ABSTRACT

Several technologies exist for improvement of cocoon including the use of natural chemicals such as plants possessing phytoecdysteroids. In the present investigation the methanol extract of the plant Pteridium aquilinum (Dennstaedtiaceae), was used in 3 concentrations (i.e. 1.25, 2.5 and 5%) dissolved in distilled water and sprayed on leaves fed on first two days of 3th, 4th, and 5th instar larvae of Bombyxmori in 3 replications of 100 insects in each replicate and a control with distilled water treatment. Results showed that, the highest silk gland weight belonged to control (0.45±0.15g), the highest cocoon weight (60.235±67.98g), cocoon shell weight (19.5±2.59g), num ber of best cocoon (53) was that of 2.5% concentration. However, 1.25% concentration showed significant differences with control and other concentrations in terms of metabolites like: cholesterol (112.16±47.46mg/dl), urea (349.37±44.78mg/dl), glucose (565.65±77.26mg/dl),LD H (17153±1014.38U/min). ALP (0.006±0.0004µmole/min/l) was recorded the highest for 5% concentration compared with control and other concentrations. However, AST and ACP did not show differences among treated and control insects. Various results were obtained on nutritional efficiency, and cocoon spinning.The possible significance of these results is discussed.

Key words: Phytoecdysteroid, *Bombyx mori*, *Pteridium aquilinum*, Sericulture

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INTRODUCTION

Sericulture is an agro-based cottage industry, combining the features of rural agriculture and industry based activities. Utilization of bio-active chemicals during silkworm rearing is one of the means of increasing production of superior quality of silk. However, the application of phytoecdysteroids, influencing the growth and development in silkworm, to improve productivity is a new concept in sericulture [7].Ecdysone and juvenile hormone (JH) are the two major circulating hormones in insects which control majority of growth and developmental activities [36].Exogenous application of the analogues or mimics of these hormones could induce derangement in the metabolic activities and create disruptions in the normal insect development. More than the titre of these hormones, the balance between these two due to a well programmed interplay decides the pattern of physiological activities in insects at any given point of time[7].Plant-produced insect molting hormones, termed phytoecdysteroids (PEs), function as plant defenses against insects by acting as either feeding deterrents or through developmental disruption[51]. But the response of silkworm, *Bombyx mori* L. to minute quantities of these hormones or its analogues is beneficial. In China, various plant sources were identified which contained moderate to high amounts of

PEs and used them in sericulture to manage the silkworm rearing during the last stage of larval development[63,8]. The major objective of using PE in sericulture is to hasten the larval maturation events in the final larval instar and to synchronize the cocoon spinning process so that the larvae can be transferred early and together to the cocoon spinning device and the larvae would form cocoons almost simultaneously. This can save a lot of skilled labor which is otherwise required to pick up only the ripe worms in time and substantial amount of mulberry leaf [45]. For such a result, PEs are usually administered at the last phase of the final instar when a handful of larvae are ripe [39].*Pteridium aquilinum*, commonly known as the western bracken fern, has proven itself to be a very resilient plant. It is found globally in a wide variety of climates and soil types, succeeding even in disturbed areas[39].Evidence from fossils suggests that the *P. aquilinum* has had over 55 million years to evolve and cultivate advanced anti-disease and anti-herbivore mechanisms [37].*Pteridium aquilinum* competes for soil nutrients and water with other species effectively and often invades disturbed areas[11]. As the *P. aquilinum* establishes itself, it impacts much of the plant life around it.Both bracken rhizomes and leaves were used as fodder for domestic animals. In Wales, shredded dried leaves mixed with straw or hay are given to horses and mules pulling trams during winter. Leaves were also given to rabbits[38]. *Pteridium aquilinum(Denstaedtiacae)* is one the PE sources[48]. In medicine, the rhizome of this plant is used as a mixture with some other plants, this mixture is fomented on the body of persons suffering from leprosy and also in paralytic patients. This hot mixture is effective in treating various kinds of skin diseases as well [27]. Silk fibroin (SF) is a natural fibrous protein spun from *Bombyx mori* silkworm. The cocoon of the silkworm is mainly composed of sericin and fibroin. Sericin is a glue-like protein that holds SF fibers together in the cocoon case. SF is composed of a repetitive sequence of amino acids: glycine, alanine and serine, and—as all fibrous proteins—is not soluble in water due to its high concentration of hydrophobic amino acids [2].Compounds extracted from plants or their derivatives may affect insect physiology in many different ways [20, 53].Generally plants produce numerous secondary metabolites out of which insect molting hormones form a major group of chemicals. It has been clearly established that most of common phytoecdysteroids with 20-hydroxyecdysone-like activity affect insect growth and development on ingestion. There are many types of ecdysteroids in plants, the most common being 20-hydroxyecdysone (20E), the amount of which varies among plant species. Because of abundant occurrence in plants, phytoecdysteroids form an important and cheaper source for commercial application in sericulture. Their molting hormone activity induces different responses in silkworms which can be manipulated for maximum benefit like early and uniform spinning behavior, increase in silk yield, enhancing productivity and reducing crop losses. The most advantageous use of phytoecdysteroids is induction of uniform spinning behavior which will not only make the management of mounting easier but because of harvesting and marketing at appropriate time, the cocoon quality will be improved with the labor becoming more scare even in developing countries, any labor saving method improves productivity and with the added advantage of the improvement in quality of cocoons. This method will be a boon to rearers. Hence it can be concluded that the application of phytoecdysteroids in sericulture under Iran conditions has a promising future.An important part of feeding ecology is the evaluation of feeding indices or efficiency indices illustrated byWaldbauer[62]. These indices demonstrate the digestion efficiency or utilization of diet or diet ingredients and in fact illustrate the conversion of food to the biomass of insects. These indices can provide valuable information about the positive or negative impact of ingredients or total food[9]. The studies on feeding indices after feeding on secondary metabolites can help to determine whether a chemical compound is an antifeedant or toxic after feeding[31]. The general feeding indices used are: approximate digestibility (AD), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), relative growth rate (RGR) and relative consumption rate (RCR). In the present investigation the effect of methanol extract of *P. aquillinum* has been investigated on growth and feeding physiology of silkworm *B. mori* in the hope of finding that this extract doesn't have any negative impact on the growth of the larvae and can be useful in improving the commercial parameters in cocoons of silkworms and ripening.

MATERIALS AND METHODS

Experimental insects

Disease free egg of bivoltine silkworm hybrid, 153×154 were used in this investigation, they were reared at 26±2˚C temperature, 75±5% humidity, under 12: 12 (light: dark) photoperiod, the moisture and temperature dependent on the age of the larvae. 'SHIN ICHINOSE (SI)' mulberry variety leaves, harvested from a frequently irrigated mulberry garden were provided to silkworms three times a day. After the second ecdysis, larvae were divided into four groups and reared under the same conditions.

Administration of Plant extract

Plants were collected from growing locations in Guilan University campus that is located in north of Iran. Briefly 10 g of dried herb powder was mixed with 100 ml methanol, the extract of this stage was concentrated in Rotary evaporator (Rotavapor RE 120 , Buchi Labortechnik AG,) and then a 10% stock solution was prepared in methanol and then three concentrations of 1.25, 2.5, and 5% was made by distilled water. This investigation used 3 replicates of 100 sampling unit in each replicate. In the experiments, 3 concentrations were used on the first 24 and 48 hrs of

third, fourth and fifth instar larvae. The solutions were sprayed on mulberry leaves before feeding and controls were treated only by distilled water and allowing leaves to lose the moisture at room temperature.

Data collection

The duration of the 5th instar was calculated in the treated larvae and control. When the larvae started ripening, the rearing beds were examined every 12 hours, the ripe worms were collected, counted and transferred to mounting frames for cocoon building. Progressive spun cocoons were calculated and plotted in column graph. After cocooning, the cocoon weight, cocoon shell weight was measured by sensitive measurer (Sartorius analytical balance, 0.0001g).

Evaluation of the effect of methanol extract on feeding indices

The following nutritional indices defined by Waldbauer[62] were calculated; approximate digestibility (AD; 100 ×[food consumption – feces]/food consumption), efficiency of conversion of digested food into body matter (ECD; 100×weight gain / [food consumption – feces]) and efficiency of conversion of ingested food into body matter (ECI; 100×weight gain / food consumption). Relative growth rate (RGR) and relative consumption rate (RCR) were defined as weight gain divided by the initial weight of the larvae and food consumption divided by the initial weight of the larvae [22].Food loses weight due to evaporation or respiration of plant material [60,62].The equation given by waldbauer[62]was used to compensate for weight in all computations. This Waldbauer equation takes into account that weight loss of food that will be consumed during the experiment, is lower than weight loss of uneaten food. The latter was determined by incubating modified leaves in petri dishes or eppendorf vials without a larvae; weight of fresh food was divided by the weight of one-day-old food, resulting in a correction factor (f). Measuring of fresh weight of the larvae was preferred to dry weights, because saving the larvae for cocooning. The nutritional indices were measured only for 2.5% concentration treatment and the Control.

Measurement of fibroin

The amount of fibroin was measured by the method of Nogueira et al. [35] 2 gram of sodium carbonate was dissolved in 1000cc of water and then to 40 cc of this solution 1 g of shell cocoons was added and was boiled for 45 min, washed with distilled water and oven dried at 45 ◦C for 48 h and then weighed. Hence the amount fibroin was calculated.

Sample preparation for enzymatic assay

Samples from each treatment were diluted with phosphate buffer (1:1 w/v) and centrifuged for 5 min in 10000 rpm. The supernatant was transferred to new tubes and was stored at -30˚C until used. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using Thomas' procedure[58].Method of Bessy et al. [6] was used for measuring Alkaline phosphatase (ALP) and Acid phophatase(ACP). The reaction mixture was 100 µl of universal buffer, 30 µl of related substrate (Acidic for ACP, Alkalic for ALP) and 20 µl enzyme. This mixure was incubated for 30 min, after that 50 µl NaOH (1M) was added. Asorbance was read and was measured based on the Biuret's method as was described by Reinhold [42] using a protein assay kit (Biochem.Co, Iran) and measuring the absorbance at 540 nm. Lactic dehydrogenase (LDH) activity (nmol of pyruvate reduced/min/mg protein) was measured according to the method of Anon [4]. Glucose was analyzed as described by Siegert [56]and total cholesterol as was described by Richmond [43] hydrolyzing cholesterol esters with cholesterol oxidase, cholesterol esterase and peroxidase. Uric acid contents in the medium were determined using uricase as described by Valovage and Brooks [61]at 500 nm. Urea was measured with urease-GDH kit (Biochem. Co, Iran) at 340 nm following the manufacturer's protocol.

Stastical analysis

All data were analyzed using SAS software and Tukey's studentized range (HSD) test in a complete randomized design [44].

RESULTS

Commercial parameters

The data on the number of best cocoon and middle cocoon characters are presented in Table 1 and Table 2. The results showed that the commercial parameters of best cocoons for the larvae treated with 2.5% extract has been improved .The cumulative spun cocoons of the extract treated larvae against the control has been depicted in Figure 1. The larvae treated with 2.5% extract had more synchronizing than the control in cumulative maturation. Anterior, middle and posterior silk gland weight in control were more in comparison to treatments (Table 3).

Table 1:Effects of three concentrations of *Pteridium aquillinum* extract on the economic traits of best cocoons of silkworm, *Bombyx mori* (Hybrid: 153×154) when administered at 1 and 2 day old of 3th, 4th, 5th instar.

concentration	Number of best cocoon	Best cocoon weight	Best cocoon shell weight	Number of best male cocoon	Best male cocoon weight	Best male cocoon shell weight	Number of best female cocoon	Best female cocoon weight	Best female cocoon weight
1.25 %	34± 0.04 b,c	39.9± 0.64 b	9± 0 b,c	22.46± 0.49 a,b	17.5± 0.33 a,b,c	2.07± 0.004 b,c	22.46± 0.45 a,b,c	7.5± 0.96 a	3.99± 0.96 a,b
2.5 %	53± 1.3 a	60.23± 1.62 a	19.5± 0.6 a	29.77± 0.59 a	24± 0.88 a	4.13± 0.45 a	29.77± 0.5 a	9.5± 0.55 a	5.17± 0.55 a
5 %	43± 0.57 a,b,c	51.62± 1.06 a,b	15± 0.00 a,b	27.04± 0.58 a,b	21± 0.57 a,b	3.53± 0.02 a,b	27.04± 0.53 a,b	7± 0.04 a	4.84± 0.04 a
Control	33± 0.88 c	39.53± 0.48 b	13.75± 0.65 a,b	12.96± 0.79 b	11± 0.08 b,c,d	3.34± 0.007 a,b	14.19± 0.96 b,c,d	8.25± 0.01 a	2.83± 0.007 a,b,c

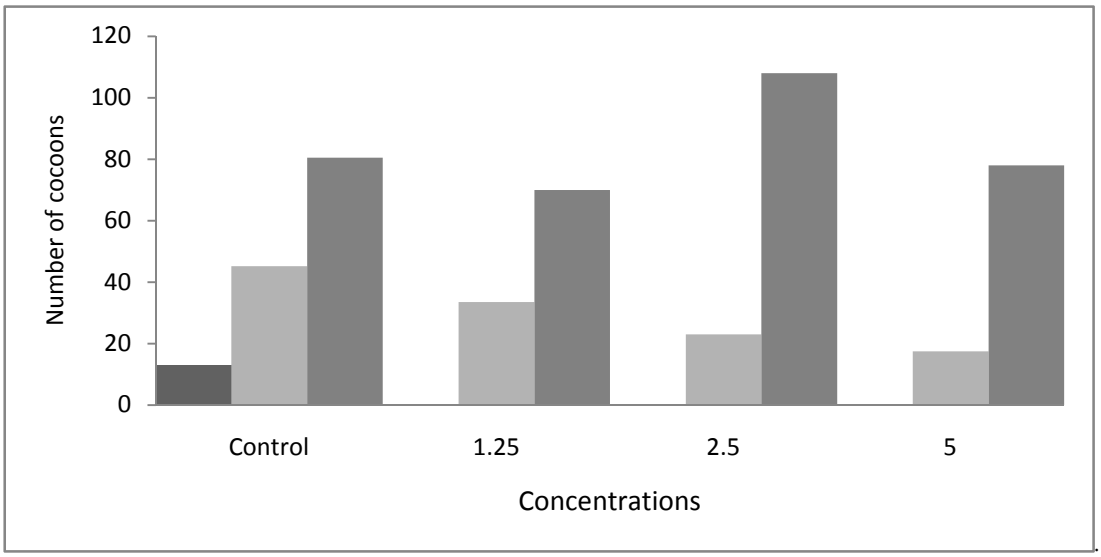
Within columns, followed by a same letter do not differ significantly.

Table 2:Effects of three concentrations of *Pteridium aquillinum* extract on the economic traits of middle cocoon silkworm, *Bombyx mori* (Hybrid: 153x154) when administered at administered at 1 and 2 day old of 3th, 4th, 5th instar.

Concentration	No. of middle cocoon	Middle cocoon weight	Number of male middle cocoon	Male middle cocoon weight	Male middle cocoon shell weight	Number of female middle cocoon	Female middle cocoon weight	Female middle cocoon shell weight
1.25 %	6.5± 0.0 6 a,b	6.91± 0.848 a,b	1.5± 0.0 88 b,c	2.525± 0.349 a,b,c	0.51± 0.0 94 b,c	2.5± 0.0 66 a	6.3± 1.053 a	0.56± 0.097 a
2.5 %	4.5± 0.0 8 a,b	7.78± 1.0 64 a,b	1.5± 0.0 88 b,c	0.5± 0.0 11 b,c	0. 5± 0.04 b,c	2.5± 0.0 88 a	2.745± 0.0 3 a	0.46± 0.0 76 a
5 %	9± 1.0 5 a	10.22± 1.5 a	5.5± 0.0 6 a,b	5.46± 0.0 6 a,b	1.1± 0.0 42 a,b	3± 0.0 77 a	3.72± 0.0 6 a	0.74± 0.0 34 a
Control	10± 0.0 8 a	10.63± 0.68 a	4.5± 0 b,c	5.42± 0.08 a,b,c	0.81± 0.09 a,b,c	1.5± 0.0 7 a	1.87± 0.0 02 a	0.3175± 0.0 5 a

Within columns, followed by a same letter do not differ significantly.

Figure 1. Effect of oral administration of *Pteridium aquilinum* extract on the hastened maturation events and synchronized cocoon spinning in silkworm, *Bombyx mori* . (Hybrid: 153 x 154). *Pteridium aquilinum* extract was administered at the two first days of 1th, 2rd, 3rd instar larvae.



Within columns, followed by a same letter do not differ significantly.

Table3: Effects of three concentrations of *Pteridium aquillinum* extract on silk gland weight of *B. mori* when administered at 1 and 2 day old of 3th, 4th, 5th instar.

treatment	Anterior silk gland weight	Middle silk gland weight	Posterior silk gland weight
1.25%	0.09± 0.0024b	0.58± 0.03b	0.25± 0.003b
2.5%	0.04± 0.01b	0.46± 0.003b	0.2± 0.002b
5%	0.1± 0.02b	0.60± 0.003b	0.28± 0.001b
Control	0.22± 0.00a	0.7± 0.005a	0.38± 0.003a

Within columns, followed by a same letter do not differ significantly .

Physiological parameters

Some physiological aspects after treating with the extract have been shown in Table 4. Results showed the amount of cholesterol, urea, Glucose and LDH was the highest for 1.25% extract and there was no significant differences between control and other treatments while the amount of uric acid was the highest for control, ALP was the highest in 5% extract.

Nutritional indices

Parameters in nutritional indices like AD and RCR indices were significantly higher in control compared to treatments. ECI, ECD and RGR were significantly higher in treatments compared with the control (Table 5).

Table5: Efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD) and approximate digestibility (AD) of B. mori when administered at 1and 2 day old of 3th, 4th, 4th instar. (T=treated , C=contro

Nutritional indices	RCR (mg/mg/day)	RGR (mg/mg/day)	ECI (%)	ECD (%)	AD (%)
Control	2490±3.5a	2807.84±4.1b	118.81±4.2b	211.15±1.2b	56.27±0.23a
Treatment	2480.43±2.7b	2928.16±3.4a	125.33±3.2a	231.45±0.24a	54.15±0.3b

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Table4: Effects of three concentrations of *Pteridium aquillinum* extract on some biochemical compounds of *B. mori* when administered at 1 and 2 day old of 3th, 4th, 4th instar.

Concentration	Cholesterol(mg/dl)	Urea(mg/dl)	Protein(mg/dl)	Uric acid(mg/dl)	Glucose(mg/dl)	LDH(IU/L)	ALT(IU/L)	AST(IU/L)	ALP(IU/L)	ACP(IU/L)
1.25%	312.16 ±47.47 a	349.39 ±44.75 a	0.007 ±0.001 a	7.57 ±0.52 b	565.65 ±77.26 a	17153.33 ±1014.38 a	1028296 ±409244.6 a	1055788 ±87042.95 a	0.001 ±0.0006 b	0.003 ±0.0007 a
2.5%	156.41 ±37.13 b	0.19 ±0.08 b	0.003 ±0.0003 a	2.97 ±0.69 b	39.33 ±21.86 b	2813.33 ±1408.98 b	138906.8 ±69634.02 a	152411.6 ±151688.7 a	0.002233 ±0.000393 b	0.005867 ±0.0004 a
5%	140.17 ±8.23 b	0.64 ±0.27 b	0.003 ±0.0005 a	12.18 ±1.55 b	126.42 ±103.09 b	4960 ±70.23 b	334244.4 ±51409 a	98392.29 ±76277.49 a	0.0069 ±0.000436 a	0.58 ±0.58 a
Control	49.65 ±27.11 b	0.3 ±0.11 b	0.003 ±0.001 a	336.57 ±60.92 a	10.25 ±2.53 b	3393.33 ±1720.09 b	814630.1 ±182454.7 a	718167.2 ±64765.08 a	0.002 ±0.0002 b	0.005 ±0.002 a

Within columns, followed by a same letter do not differ significantly .

DISCUSSION

The cumulative spun cocoons of *pteridium aquillinum* extract treated larvae showed more synchronizing for 2.5 % extract and other concentration compared with the control. This sort of a reduction in mounting duration in the extract administered silkworms reduces labor involvement in picking of the ripe worms and saves mulberry leaves. A similar result was reported by using PE extracted from *Sesuvium portulacastrum* on silkworm hybrids [33]and that from Caryophylaceae family of plants on pure silkworm breeds[59]. This difference in the larval and mounting duration is because of physiological role played by the exogenous ecdysteroid on the insect development system. The feeding larvae always contain a baseline level of ecdysone but reaches to pupation in reducing peak before pupation[47]. By giving an extra dose of plant based ecdysteroid content in silkworm is advanced and thereby change the larval behavior as such.The exogenous amount of PE also affects the larval weight, cocoon and shell weight as evident from Table 1 and 2. But the changes in these traits in response to PE treatment are dependent on the time of the treatment. Chow and Lu [8] reported when plant based ecdysteroid was administered to silkworm at early stages of 5th instar. The response of silkworm varied substantially depending on the time of treatment. The change in the reduction in the larval duration was inversely proportional to the cocoon characters as seen in the present study. These manifestations give an impression that PE administered early in 5th instar induces juvenoid like response in silkworm. Juvenoid treatment prolongs the larval period and increases the cocoon/silk weight in silkworm, *B.mori*. It was reported that silkworms are sensitive to exogenous ecdysteroid when administered at different hours [47]. But such sensitivity and the related manifestations largely depend on the time of applications. Dai et al. [12] indicated that ecdysone plays a significant role in nucleic acid metabolism and the related protein synthesis in silkworm. Although it induces growth and silk production, depending on the age of administration the intensity of manifestation will be different. According to Chow and Lu[8] PE administered up to 48 h of 5th instar had induced an enhancement in the silk synthesis and the cocoon weight increased considerably. But the administration after 72 hours of 5th instar reduced the feeding period notably with an obvious reduction in the cocoon and cocoon shell weight. But the treatment at the onset of spinning brought about acceleration of maturation events and synchronization of spinning without a significant decrease in the quality of cocoons. The result of the present study agrees with these observations. The 2.5 % treatment is done obviously to save a silkworm crop from an imminent loss either due to an unforeseen shortage of mulberry leaves or disease outbreak, in the last phase of silkworm rearing. The treatment brings about a reduction of about 24 hours in the fifth instar period although it certainly reduces the economic traits as well. But the reduction in the cocoon crop is offset in this context because the crop would have been lost fully had the treatment been not made. In such event, the reduction in the cocoon traits is understandable since the final instar larvae do not get enough time to consume the leaves to the potential or as per its general requirement and thus the conversion to the silk protein is quantitatively affected though not qualitatively. Here the thrust was more on the hastening of maturation process rather than attaining any improvement in the silk related economic traits. Hastened maturation and synchronized cocoon spinning save good amount of mulberry leaf and labor apart from enabling the farmer to market the produce in a single lot. Earlier, Shivakumar et al. [54,55]reported such an effect on silkworm when extracts of plants such as a physiological explanation for the varied effects of PE on silkworm could be in the following line: As proposed by Fukuda [23], the increase in silk gland function during feeding period of the last larval instar is due to stimulation by ecdysteroid. For this, the origin of ecdysteroid need not necessarily be endogenous. It was also understood that the response of the silk glands to exogenous ecdysteroid depends on the developmental stage of the larvae[47]. Ecdysteroid represents a stimulator of silk gland. Feeding larvae always contain low level of ecdysteroid that may be indispensable for development [46].. The dependence of silk production on ecdysteroid possibly reflects a general tissue requirement for such a low level of ecdysteroid concentration. A rise of ecdysteroid titre towards the end of fifth instar apparently terminates feeding and stimulates cocoon spinning in preparation to the metamorphosis. For proper course of these developmental events, it is significant that the ecdysteroid level is slightly elevated for 1-2 days before rising to the molt inducing height. The elevated titre of ecdysteroid apparently shifts silk glands to their regression phase when they reach maximum protein synthesis. The treatment, 5 % just act this way and advances the molt inducing peak early [45]. Keshan and Ray[29] reported the increase in cocoon shell weight after the low dose of estradiol 17β was not due to the extension of feeding period as juvenile hormone treatment that induced a prolongation of the instar that caused increase in silk production[29].Therefore the increase of cocoon shell weight after estradiol treatment seemed to be the consequence of increased cellular activity of the silk gland [30].that may be this event is occurred for the results are brought in table 1 and 2.Different factors may affect enzymatic and non-enzymatic processes in insects such as: climate, diet, chemicals, etc. The amount of Cholestrol, Urea, Glucose and LDH didn't significantly differ in 2.5 and 5 % concentration of the extract with control but in 1.25 % increased significantly. Etebari and Matindoost [17] reported that if the feeding activity is normal, glucose and cholesterol amounts in the hemolymph of silkworms increase. When the feeding of larvae, however, is interrupted the amount of these metabolites are severely decreased [18].And this researched to this result that shows in 1.25% feeding was beter than control and other treatments, However good feeding is a positive point because can affect growth of insect and reproduction and crops will de achieved from it. Nath [34]reported that lethal and sublethal doses of ethion and fenitrothion increased the amount of

glucose and trehalose in silkworm. This could be due to imbalance in the homeostasis of the silkworm. Our results similarly point to the possibility that increase in the amount of glucose could also be due to increase in carbohydrate metabolism caused by extract stress[53]. Urea and uric acid are excreted end product of insects and their amount is correlated with the amount of protein in the insect's body. Dungern and Briegel [14] reported that the activity level of xanthin dehydrogenase increased due to Presence of higher protein in the hemolymph and our results showed the amount of protein was the most in 1.25 % that can be the reason of increased urea. The amount of uric acid decreased in the hemolymph of treated larvae. This result indicates that the decrease in uric acid in all of concentration in comparison with control hemolymph is probably due to altered metabolic pathway after treatment that prevented the natural excretion of uric acid from the insect body [19].ALP is hydrolytic enzymes that hydrolyze phosphomonoesters at alkaline pH [32]. Toxic chemicals, on the other hand, decrease the nutrition efficiency and ALP activity [64,15]. Senthil Nathan et al. [50] showed that treatment of rice plants with *Melia azedarach* Juss (Meliaceae) extracts decreased the activity level of ALP in *Cnaphalcrocis medinalis* (Guenee). These authors reported that feeding *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on *Ricinus communis* L. treated with azadirachtin decreases the amount of this enzyme in the midgut [49].Our results show that the activity level of this enzyme increases at 5 % concentration. Increasing ALP activity level may indicate perhaps that the enzyme is involved in detoxification processes. Acid phosphatase (ACP) is a lysosomal enzyme concerning with digestion of foreign substances and bacteria inside the cells[26]and is involved in the defense mechanisms of both vertebrates and invertebrates [10]In addition, the deterioration in the activity of alkaline phosphatese can occur in all types of liver diseases [21]Our result showed this extract didn't act as a stress. The activity of two amino transferases, AST and ALT, were followed. The amino transferases are important components of amino acid catabolism; which is involved in transferring an amino group from one amino acid to a keto acid[66]. The AST and ALT serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions [20]. Ender et al. [16]reported that diet with high level of methyl parathion significantlyincreased the activities of ALT and AST in greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae. Whereas the activity level of amino transferases decreased by low level of diazinon in the diet [21]. However our result showed there is no significant difference between treatments and control and it can be a reason for that *Pteridium* extract didn't act as an toxic extract [53].Evaluation of feeding indices under methanol extract of *P. aquillinum* show that approximate digestibility in larval feeding on treated leaves is decreased compared to the controls. Probably the insect tries to compensate for the low consumption. Senthil Nathan[50] reported opposite results with *Melia azedarach* on *Cnaphalocrocis medinalis* in longer larval duration, low consumption and maintaining food for a longer duration in the gut, and as a result, leading to higher approximate digestibility. Stoyenoff [57] reported that when gypsy moth larvae feed less, the food will pass slowly through the gut hence, it enhances the digestibility that is similar with our result for treating with the extract. Relative growth rate (RGR) which follows weight gain in control larvae is significantly lower than treated larvae. The lower RGR could be due to irreparable damages made to midgut lumen cellular surfaces [28]. Gusmao et al. [24] reported the discrepancy in peritrophic membrane and damages of gut cells in *Aedes aegypti* treated with methanol extract of *Derris uruca*. Low RGR with increasing concentration shows low quality of food and probably it acts as an inhibitor. Lack of specific components particularly nitrogen or water leads to a lower growth rate and lower metabolic efficiency[15] and our results showed increased RGR compared to control, hence it can be a positive point for using of this extract.As per results obtained, it can be said that *B. mori* feeding on treated food faces upper feeding indices except AD index. Senthil Nathan [50], Shekari et al. [53] and Zapata et al. [65], all reported low feeding indices after treatment of their respective insects by plant extract or essential oils and Amala Rani [3] reported upper feeding indices in larvae of *B. mori* were treated supplementation of Amway protein. Hala and Reem [25] reported that the extract of three plants namely Oshar (*Calotropis procera*), Harmal (*Rhazya stricta*) and Hargal (*Solenostemma* argel) severely reduces RGR, RCR and ECI in treated larvae of *Spodoptera littoralis.* These authors believe that the mechanisms of an antifeedant agent may be due to disruption in the cellular surfaces of the midgut. Hence, reduction of digestion as a result of covalent bands with food proteins or digestive enzymes. Unsuitable food ingredients and the food regiments lacking essential components for growth lead to a higher digestibility and consumption index but reduced RGR and ECI [9]. Lower RGR, ECI and ECD probably lead to delay in larval growth and formation of smaller pupa which have a direct relation to fecundity and longevity of the adult insect and make them susceptible to diseases and natural enemies. prutz and Dettner [41] reported for all concentrations, RCR was higher than in control than in Bt groups in *chilo partellus larvae* . Lower RCR values with respect to the control were observed in different studies dealing with the effect of microbial Bt formulations on lepidopteran larvae [5,13, 22,40,41]also reported in 10-day-old of the Bt group of *Chilo partellus* had a higher ECD and a lower AD than in the control that was similar to our results.

CONCLUSION

It is concluded that this plant extract could be a nominated plant for further investigation leading to the development of exact phytoecdysteroid for rearing this economic insect thus helping farmers with their economics.

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