Great Expectations of Garden Cress (*Lepidium sativum* L.) Seed Powder in Sericulture Industry

II. Correlation Studies between Certain Reproductive, Phenotypic, Biochemical, and Economic Parameters in *Bombyx mori* L.

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ABSTRACT

This paper is a supplementary study of a research article (under publication) entitled: "Great Expectations of Garden Cress (*Lepidium sativum* L.) Seed Powder in Sericulture Industry"- I. With a Brief View on the Positive Effects of Mulberry Leaf Enrichment with the Various Phytochemicals in *L. sativum* Seed Powder on *Bombyx mori* L. Economic Parameters. Analysis of correlation relationships between *Bombyx mori* different economic parameters revealed that of the studied 39 analyses, eight, six, and twenty-five correlation relationships proved to have a significant correlation, a marginally non-significant correlation, and a non-significant correlation, respectively. The cocoon shell weight or the cocoon shell ratio (%) showed to be a function of the pupal weight or the cocoon weight, respectively. Hence, *B. mori* pupal weight or cocoon weight is the best reliable predictor of cocoon shell weight or cocoon shell ratio (%), respectively (r=0.936-0.982 or r=0.545-0.901, in respect). The obtained negative correlation relationships could give evidence for possible trade-offs between *B. mori* female fecundity and each of the following: pupal weight, female longevity, female body size, spermatophore counts per female moth, or egg size. Also, the ANOVA and the significant negative correlation relationships in the controls indicate that *Lepidium sativum* seed powder is an influential factor in improving the *B. mori* economic parameters. Application of correlation-regression analyses in *B. mori* rearing might be advisable either to predict *B. mori* economically important parameters or to encourage for trade-off studies between life-history parameters which are important in *B. mori* rearing and selection or breeding programmes.

Key words: *Bombyx mori*, *Lepidium sativum*, correlation-regression analyses.

INTRODUCTION

Among silkworms (Bombycidae or Saturniidae), little is known about the correlation relationships between their economic parameters, although these correlations are importantly required in sericulture field for making direct or indirect contribution(s) to rearing or breeding programmes of such economically beneficial insects (*e.g.*, *Bombyx mori* L.). Also these relationships can be adopted to determine which of the sericultural parameters are more or less likely to rely on as an accurate predictor or estimator of silkworm economic parameters (Miller *et al*., 1982 & 1983; Kotikal *et al*., 1989; Baidhera, 1992; Kumar *et al*., 1998; Kasmaei & Mahesha, 2012; Mahmoud & El-Hattab, 2012 and Mahesha *et al*., 2013). Furthermore, obtaining a negative correlation between the studied parameters would give evidence for a possible trade-off between these parameters. The existence of trade-off (*i.e.*, increase of one life-history parameter at the expense or cost of another one) is important in an individual life-history to maximize one of the economically important parameter (Ellers, 1996; Eady *et al*., 2007 and Stürup *et al*., 2011). Therefore, this paper presents data on a number of correlation relationships between *B. mori* reproductive, biochemical, phenotypic and cocoon parameters aiming to shed some light upon the importance of such correlation studies on predicting and maximizing *B. mori* economic parameters that would gain ground in sericulture industry.

MATERIALS AND METHODS

L. sativum seeds used in this study were obtained from the local market of the type grown in Al-Qaseem area in Saudi Arabia, and authenticated by an expert taxonomist. The seeds were ground, by an electrical grinder, to a fine powder and stored in an airtight glass jar. Three concentrations (0.25, 0.50, and 1.00%, w/v) were prepared in distilled water. Based on the tested concentration, the calibrated weight of *L. sativum* seed powder and distilled water were thoroughly dissolved in a suitable container until getting a sort of a homogenous suspension. Fresh and clean mulberry leaves were dipped, separately, in the tested concentration for *ca.* one minute (with stirring in order to ensure a complete

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coverage of the tested suspension for the leaf-upper and lower-surface) and air-dried for 5-10 minutes.

B. mori larvae were allocated into three groups; the first group served as the control, the second and the third groups served as the treatments. Control larvae were fed (from egg hatching till cocoon spinning) on mulberry leaves treated with distilled water only. Treatment-larvae of the second group were fed (from egg hatching till cocoon spinning) continuously on mulberry leaves enriched with *L. sativum* seed powder suspension of each tested concentration (continuous feeding); whereas *B. mori* larvae of the third group were fed on treated leaves for 48-hour interval, then untreated mulberry leaves were supplied for 48-hour interval; then treated mulberry leaves were supplied for another 48-hour interval, and so on till cocoon spinning (discontinuous feeding). All the treatments and the control were replicated thrice, and each replication consisted of 100 larvae.

The observations were recorded on the following economic and physiological parameters: cocoon weight, cocoon shell weight, cocoon shell ratio percentage, pupal weight, adult longevity, female moth fecundity (number of deposited eggs per female), number of spermatophores per female moth, and egg size (*i.e.,* egg length and width; egg surface area and volume). Egg volume was estimated as a prolate spheroid, using the formula $V=4/3\ \pi\ abc$ (a;b;c are the radii at different planes; Seth *et al.,* 2004). Dimensions of the egg (a, b, c) were measured using an ocular micrometer on a dissecting binocular microscope (20X). The egg surface area was calculated using the formula of an ellipse ($A = \pi\ ab$; where a = length of semimajor axis and b=length of semiminor axis; Duplouy and Hanski, 2015). Also, the effect of body size of male and female moths, and number of matings (number of spermatophores per female) on female moth reproductive output (fecundity and longevity) were evaluated. The lengths of forewing, hind femur, or hind tibia (as indices of body size) were measured using both of a millimeter paper for scale and a dissecting binocular microscope at 20X according to Olson *et al.* (2000) and Reinhardt (2001). The total protein-, lipid- and carbohydrate-content of female moth internal reproductive tract (*ca.* 60 females per treatment) was also quantified directly after their death. The total protein, lipid, and carbohydrate contents were estimated from frozen samples (-20°C) of the female moth internal reproductive organ homogenates by using the Kjeldahl method and Soxhlet (see Sáez-Plaza *et al*., 2013 and Pombal *et al*., 2017, respectively).

The first paper evaluates the economic benefit(s) of *B. mori* larval feeding, continuously or discontinuously, on *L. sativum*-enriched mulberry leaves - which collected in comply with relevant institutional, national, and international guidelines and legislation- on the pupal- and the adult- performance. Results of the treatments and the controls were statistically analyzed using the ANOVA followed by Duncan's multiple range test at 5% or 1% probability level.

In the course of the present study, forty data were randomly selected from each *B. mori* tested parameter. The following parameters were studied: reproductive fitness parameters (moth fecundity - number of eggs laid per female moth -, spermatophore counts per female - number of matings -, moth longevity, egg size-measured as egg surface area and egg volume); phenotypic parameters - moth body size (measured as forewing, hind femur, and hind tibia lengths); biochemical parameters (the total protein-, lipid-, and carbohydrate-content in the internal reproductive tract of *B. mori* female moth at death); cocoon parameters (cocoon weight, pupal weight, cocoon shell weight, and cocoon shell ratio percentage).

Statistical Analysis

Pearson correlation and simple linear regression procedures were adopted to determine the relationships between the studied parameters. Statistical analysis were performed using the IBM SPSS Statistics for Windows, Version 20, Armank, NY, IBM Crop (2011).

RESULTS

In order to understand better how to increase the egg or/and silk yield, as important physiological events, in *B. mori* life-cycle, correlation coefficient analysis and regression were calculated for a 39-pair of sericulture economic parameters which may have significant or insignificant correlations that would be a good predictor of gaining better results in both egg and silk production, as well as for benefiting from correlation findings in achieving better rearing programmes for *B. mori.* Correlation coefficients are shown in Tables (1-3). The present data were summarized in three groups on the basis of their significance as follows: (1) a significant or highly significant group (8-pair), (2) a marginally non-significant group (6-pair), and (3) a non-significant group (25-pair). Table (1) represents data of the first group and displays the correlation coefficient (r), the determination coefficient (r^2), the exact *p*-value, the value of $F_{(1,38)}$, and sample size (n = 40). A strong, highly significant, and positive correlation was found to exist between *B. mori* pupal weight and cocoon shell weight in both the control group and the experimental groups [see Table 1 and Fig. 1(A)].

Table 1. Summary of significant positive or negative correlations between some economic parameters in the mulberry silkworm, *B. mori*

Correlation between	Correlation coefficient (r)			*p*-value			Determination coefficient (r^2)			$F_{(1,38)}$		
	Control	Treatment*		Control	Treatment*		Control	Treatment*		Control	Treatment*	
		c	d		c	d		c	d		c	d
Pupal weight and cocoon shell weight	0.936	0.982	0.980	< 0.001	< 0.001	< 0.001	0.875	0.964	0.961	267.044	1023.240	936.204
Cocoon weight and cocoon shell ratio (%)	0.545	0.901	0.894	< 0.001	< 0.001	< 0.001	0.297	0.811	0.799	16.025	162.967	151.044
Pupal weight and fecundity	– 0.323			0.042			0.105			4.441		
Female moth longevity and fecundity	– 0.345			0.029			0.119			5.147		
Female moth hind tibia length** and fecundity	– 0.352			0.026			0.124			5.374		
Female moth hind tibia length** and total carbohydrate content*** at death			0.324			0.041			0.105			4.467
Fecundity and egg surface area	– 0.343			0.030			0.118			5.064		
Spermatophore count and fecundity	– 0.333			0.036			0.111			4.749		

*c; d: Continuous and discontinuous larval feeding on mulberry leaves-enriched with *L. sativum* seed powder, respectively.

**As an index of body size.

***The total carbohydrate content in the internal reproductive tract of *B. mori* female moth at death.

Sample size (n) = 40.

It obviously appears that *B. mori* pupal weight is the best estimator for cocoon shell weight (r=0.936, 0.982, and 0.980; r^2=0.875, 0.964, and 0.961, in respect, $P<0.001$; Table 1). Hence, *B. mori* cocoon shell weight as a function of pupal weight could be estimated by the equation of the simple linear regression shown in Fig. 1(A).

Another strong, highly significant, and positive correlation (r = 0.901 and 0.894; r^2 = 0.811 and 0.799, respectively, $p<0.001$) was recorded between cocoon weight and cocoon shell ratio (%) for the two patterns of *B. mori* larval feeding *viz.*, the continuous and the discontinuous. As seen, their two correlation coefficients were nearly similar (*i.e.*, r $\simeq$ 0.90); whereas their corresponding value for the control group was reflecting a moderately strong, highly significant positive correlation (r =0.545, r^2 =0.297, $p<0.001$) between the related two parameters. However, this highly correlated trend (Table 1) reveals that *B. mori* cocoon weight can be used as a predictor of *B. mori* cocoon shell ratio% by adopting the simple linear regression equation displayed in Fig. 1(B).

Weak, but significant, negative correlations between fecundity and each of the following parameters: pupal weight, female longevity, female hind tibia length (as an index of body size), egg surface area, or spermatophore count per female moth, were present in the control group. The correlation coefficients (r) of these relationships ranged from – 0.323 to – 0.352, and their exact *p*-values varied from 0.026 to 0.042 (Table 1). In the treatment group, *viz*. the discontinuous larval feeding pattern, the female hind tibia length was correlated with the total carbohydrate content in the internal reproductive tract of *B. mori* female moth at death. This correlation was significant, weak and positive (r= 0.324, p = 0.041; Table 1). As shown in Table(1)and Figures (2-5), such significant, positive or negative, weak correlations (r^2 ranged between 0.105 and 0.124) may provide a relatively good predictor of fecundity in *B. mori* female moths. Also, at discontinuous larval feeding pattern, *B. mori* female moth body size (measured as hind tibia length) was found to be weakly and negatively or positively correlated with either fecundity or the total carbohydrate content in the female moth internal reproductive tract at death, respectively (Table 1 and Figs 3(B) and 4(B)). Table (2) represents the findings of the second group, *viz.*, marginally non-significant correlations, and displays values of correlation-regression analysis (*i.e.*, r, r^2, *p*-value, and $F_{(1,38)}$). In the control group there was a marginally non-significant, weak, and negative correlation between *B. mori* female moth fecundity and egg volume (p = 0.074, r = -0.285, r^2 = 0.082, $F_{(1.38)}$ = 3.372). It means that as the female fecundity decreases, the egg volume tends to increase and *vice versa*. A similar correlation was detected between the female hind femur length (as an index of body size) and both egg surface area (p = 0.095, r = -0.268, r^2 = 0.072, $F_{(1.38)}$ = 2.934; in the treatment group-continuous larval feeding pattern) and the total lipid content in the female moth internal reproductive tract at death (p = 0.087, r = -0.274, r^2 = 0.075, $F_{(1,38)}$= 3.081; in the control group). Also, a marginally non-significant, weak and positive correlation was recorded between female hind tibia length and female fecundity (p = 0.077, r = 0.283, r^2 = 0.080, $F_{(1.38)}$= 3.312; in the treatment group-discontinuous larval feeding pattern). A similar correlation was observed between *B. mori* male moth body size (measured as hind tibia length) and spermatophore count per female (p = 0.055, r = 0.306, r^2 = 0.094, $F_{(1,38)}$ = 3.928; in the treatment group-continuous larval feeding pattern). It means that as *B. mori* female or male moth body size (measured as hind tibia length) increases the female fecundity or the spermatophore count per female, in respect, tends to increase, and *vice versa*. The correlation between female body size (measured as forewing length) and number of eggs laid per female (fecundity) was marginally non-significant, weak, and negative (p = 0.094, r = -0.269, r^2=0.072, $F_{(1:38)}$= 2.958; in the treatment group- discontinuous larval feeding pattern; Table 2). It means that the body size of *B. mori* female moth was somewhat negatively correlated with her fecundity. In general, the female body size (measured as forewing length or hind tibia length) was somewhat, significantly or marginally non-significant, correlated with her fecundity (Tables 1 and 2). A similar correlation was also seen between female moth body size (measured as hind tibia length. or hind femur length) and the total carbohydrate or lipid content in her internal reproductive tract at death, in respect (Tables 1 and 2). On the other hand, the male body size (measured as hind tibia length) was somewhat positively correlated with mean spermatophore counts per female (Table 2). Also, of the 39- correlation analysis between the studied parameters of *B. mori*, 25-analysis showed non-significant correlations. Results of these analyses are summarized in Table (3).

DISCUSSION

In rearing or breeding managements of the bombycid *B. mori* for gaining better yields of silk and eggs, selection for fit male or female moths and the availability of highly nutritious and cheap larval diets are so important in order to achieve the commercially desired improvements in sericulture area.

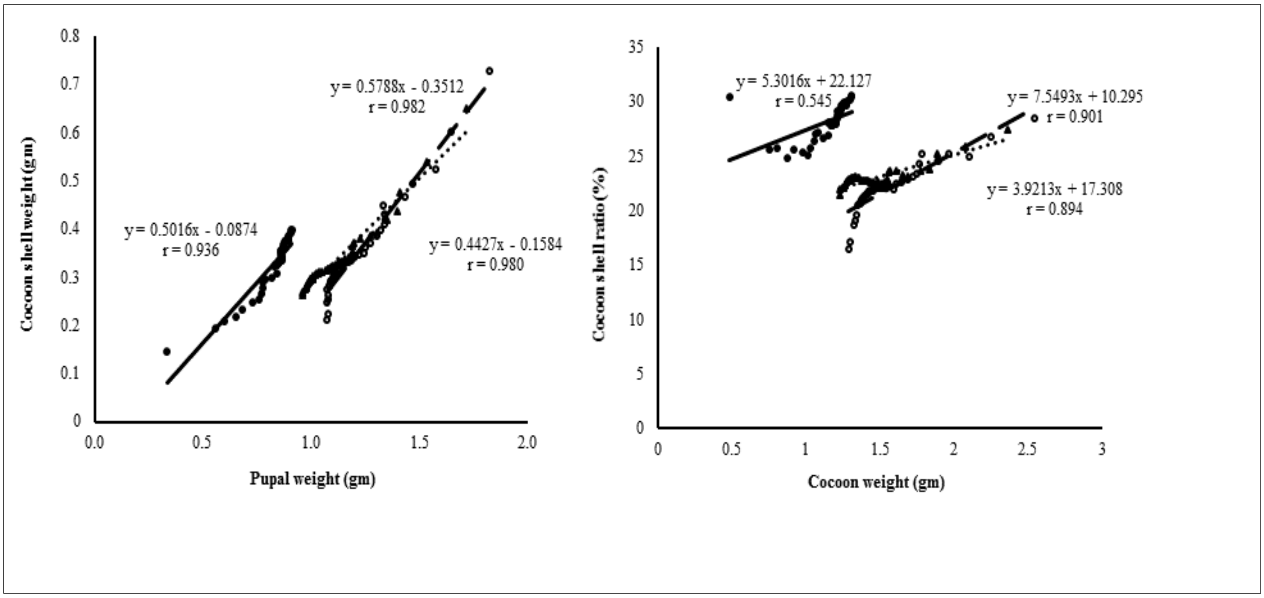

Fig. 1. Relationship between cocoon shell weight and pupal weight (A) and between cocoon shell ratio (%) and cocoon weight (B) in both treatment groups [*B. mori* larval feeding continuously (c; ▪ ▪ ▲▲▲) or discontinuously (d; ⋯⋯ □□□)] on *L. sativum* seed powder-enriched mulberry leaves and the control group (▬ •••)

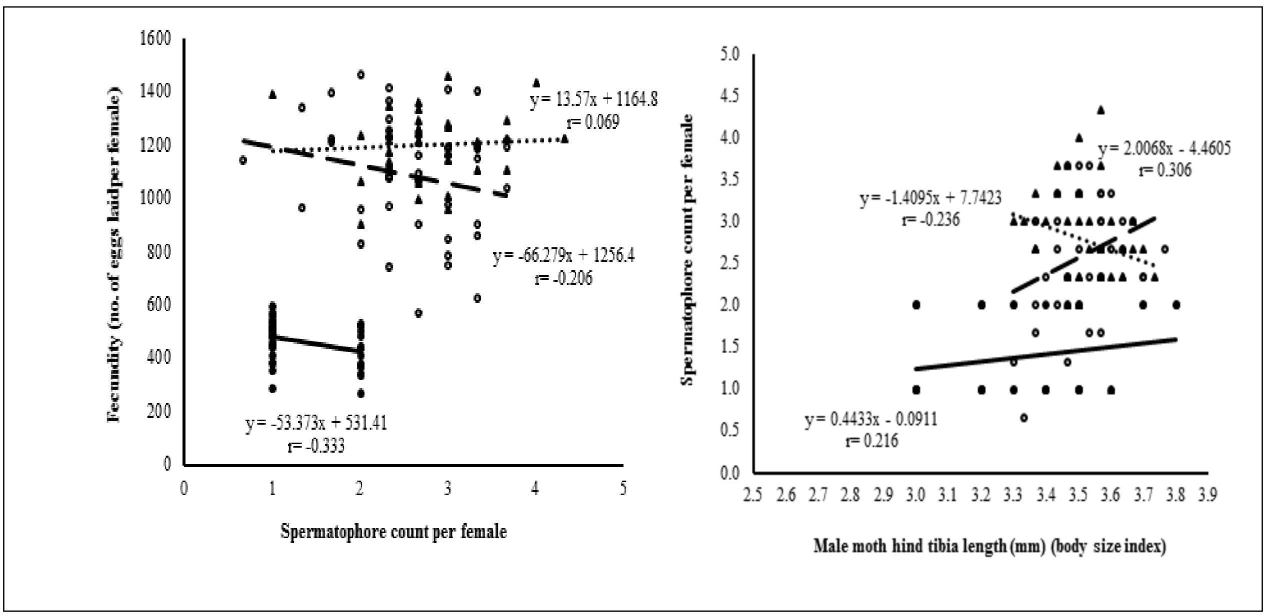

Fig. 2. Relationship between spermatophore count per *B. mori* female moth and male moth hind tibia length (body size index) (A) or female fecundity (B) in both treatment groups [*B. mori* larval feeding continuously (c; ▬ ▬ ▲▲▲) or discontinuously (d; ⋯⋯ □□□)] on *L. sativum* seed powder-enriched mulberry leaves and the control group (▬ •••)

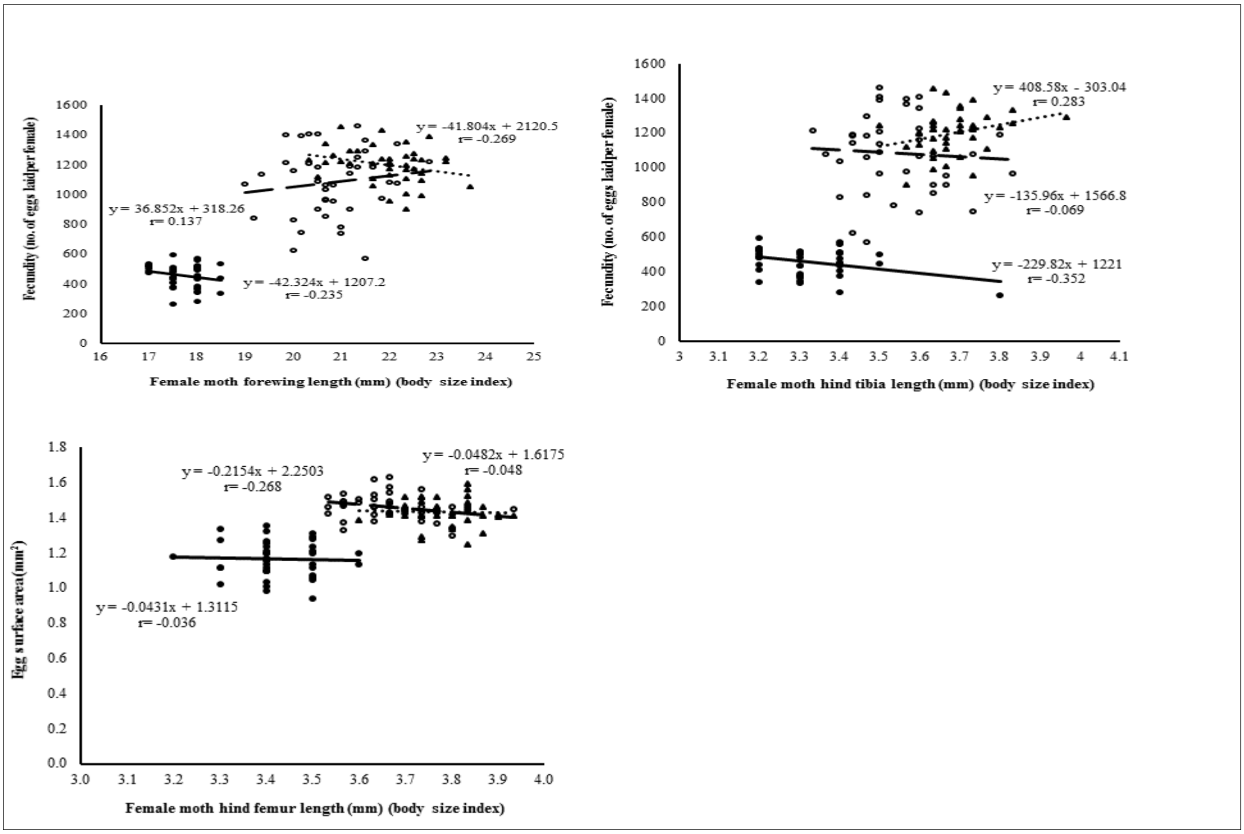

Fig. 3. Relationship between *B. mori* female moth body size, as measured by her forewing (A), hind tibia (B) or hind femur (C) length, and her fecundity or egg size (egg surface area), respectively, in both treatment groups [*B. mori* larval feeding continuously (c; ▬ ▬ ▲▲▲) or discontinuously (d; ⋯⋯ □□□)] on *L. sativum* seed powder-enriched mulberry leaves and the control group (▬ •••)

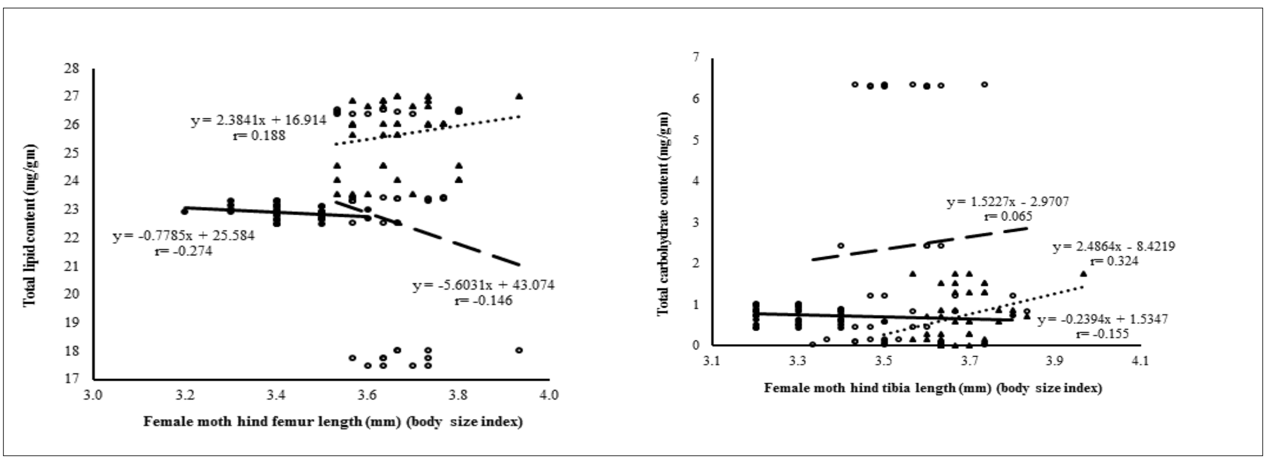

Fig. 4. Relationship between *B. mori* female moth body size, as measured by her hind femur (A) or hind **tibia (B) length, and the total lipid or carbohydrate content in her internal reproductive tract at death, respectively, in both treatment groups [*B. mori* larval feeding continuously (c; ▬ ▬▲▲▲) or discontinuously (d; ·· □□□)] on *L. sativum* seed powder-enriched mulberry leaves and the control group (▬ •••)**

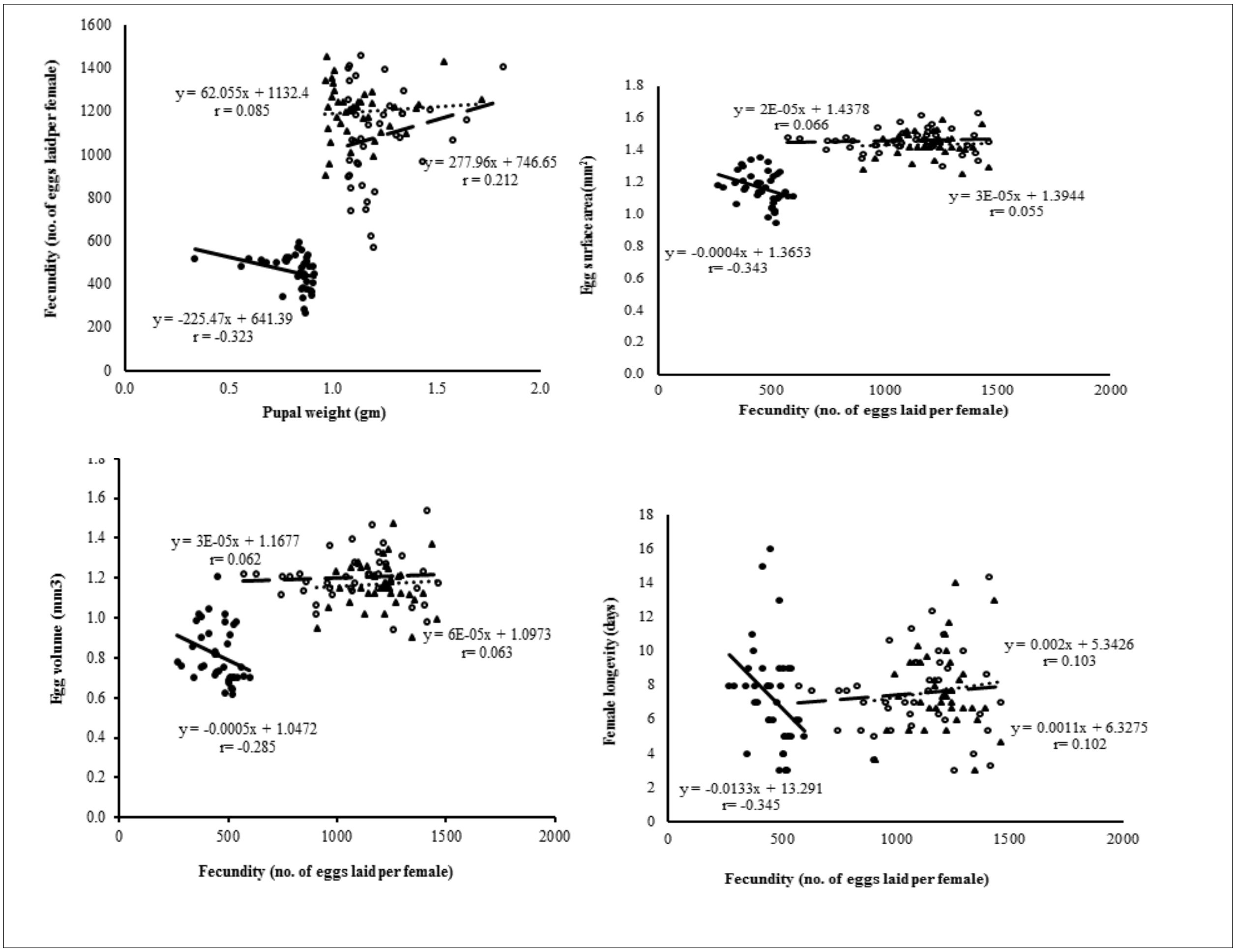

Fig. 5. Relationship between *B. mori* female moth fecundity and pupal weight (A), egg size, as measured by egg surface area (B) or egg volume (C), as well as her longevity (D), respectively, in both treatment groups [*B. mori* larval feeding continuously (c; —— ▲▲▲) or discontinuously (d; ······ □□□)] on *L. sativum* seed powder-enriched mulberry leaves and the control group (— •••)

In this concern, few attempts have been carried out to estimate the relationships between the economically important parameters of *B. mori* which might be act as a good predictor or estimator of egg or silk production and *B. mori* performance as a whole (Shamachary *et al.*, 1980; Miller *et al*., 1982; Singh & Prasad, 1987; Kotikal *et al*., 1989; Baidhera, 1992; Ghosh *et al*., 1996; Singh & Kumar, 1996; Kumar *et al*., 1998; Kasmaei & Mahesha, 2012 and Mahesha *et al*., 2013). 39 pairs of sericulture economic parameters were subjected to correlation and regression analyses to determine both the type of correlation (*viz*., positive or negative; significant, non-significant, or marginally non-significant as well) and the degree or strength of the correlation (*viz*., strong or weak). *B. mori* pupal weight showed a strong and highly significant, positive correlation with the cocoon shell weight (r=0.98); hence, the pupal weight appears to be the best reliable estimator of cocoon shell weight in *B. mori*. In other words, the *B. mori* cocoon shell weight, as a function of pupal weight, could be estimated by the equation of the simple linear regression. Another similar correlation trend has been observed between cocoon weight and cocoon shell ratio (%) where the cocoon weight could provide a very good prediction (r=0.90) for *B. mori* shell ratio (%) by adopting their simple linear regression equation. A similar result has been reported by Mahesha *et al.* (2013). Also, in the treatment group, there is a weak positive relationship (r=0.32) between the female moth body size (measured as hind tibia length) and the total carbohydrate content in her internal reproductive tract at death (see Table 1). As *B. mori* moths do not feed, the latter result suggests that carbohydrate availability in *L. sativum* seed powder (*ca*., 34-87%;

see, for example, Gokavi *et al*., 2004; Wadhwa *et al*., 2012; Berehe & Boru, 2014 and Solomon *et al*., 2016) is responsible for the positive correlation relationship between *B. mori* female moth body size and carbohydrate reserves in her internal reproductive tract at death. Thus, it seems that presence of *L. sativum* seed powder in *B. mori* larval feeding diet (mulberry leaves) could provide an excellent energetic resource for *B. mori* female moth fitness (data under publication-paper I-; see also Rivero and West, 2002).

Additionally, *B. mori* female moth hind tibia length, as an index of body size (Ruttner *et al*., 1978; Ward & Simmons, 1991; Honěk, 1993 and Rivero & West, 2002) was found to be weakly and negatively correlated with her fecundity (r = - 0.35) in the control group. However, in the treatment group, there was also a weak, but marginally non-significant, positive correlation (r=0.28) between *B. mori* female moth body size (measured as hind tibia length) and her fecundity. The lack of a significant correlation in this relationship was unexpected and might indicate that the used sample size for Pearson correlation analysis was small (n= 40) to detect accurately this correlation. Such a relationship between female moth body size and fecundity has an intercept below zero (y = 408.58x-303.04), this suggests that the female moth-reproductive output will increase with body size (see Karlsson and Wickman, 1990). Such an explanation might be accepted to boost up the present suggestion. Further, the female body size is usually a good predictor of her fecundity (see Honěk,1993). In lepidopteran species other than *B. mori*, the female body size/fecundity relationship has been reported by several authors, where this relation is direct or indirect (Cook, 1961; Wiklund & Karlsson, 1984; Haukioja & Neuvonen, 1985; Wiklund *et al*., 1987; Leather, 1988; Wickman & Karlsson, 1989; Karlsson & Wickman, 1990; Marshall, 1990; Honěk, 1993 and Gage, 1998). Hence, the present findings may also suggest a possible trade-off between *B. mori* female moth fecundity and her body size. This indicates that there is a physiological cost of being a highly fecund moth in *B. mori* which might be compensated by *B. mori* larval feeding on a well-nourished diet (*L. sativum*-enriched mulberry leaves).

Also, four weak, but significant, negative correlations were observed between *B. mori* female moth fecundity and pupal weight (r = - 0.32), female moth longevity (r = - 0.35), egg surface area (r = - 0.34), and spermatophore counts (repeated matings) per female moth (r = -0.33). These negative correlations may reflect other possible trade-offs between *B. mori* female moth fecundity and the aforementioned four life-history parameters. In the literature, *B. mori* heavy pupae result in high fecundities, and a highly significant positive correlation (r=0.84-0.92) between pupal weight and fecundity has been recorded (Govindan *et al.,* 1990; Jayaswal *et al.,* 1991; Singh, 1994 and Singh & Kumar, 1995). On the contrary, in the present study, *B. mori* pupal weight, in the control group, is negatively correlated with fecundity. However, the ANOVA of the first part of this paper (under publication) reveals that heavier pupae, in the traetment group, tend to produce highly fecund female moths which laid significantly more and large eggs (measured as egg surface area, *i.e.*, germ band region in the periplasm, and egg volume, *i.e.*, quantity of yolk; see Campbell,1962) than do those in the control group. Also, in the literature, there is evidence of correlations between pupal weight and fecundity in other lepidopteran species (Danthanarayana, 1975; Marks, 1976; Hough & Pimentel, 1978; Miller *et al.,* 1982 & 1983; Proshold *et al.,* 1982 and Gilbert, 1984). On the other hand, a weak but marginally non-significant (*p*=0.07) negative correlation (r = -0.29) was found between *B. mori* female moth fecundity and egg volume. Thus, *B. mori* egg size (measured as egg surface area or volume) as a function of female moth fecundity provides a weak estimate. In other words, female moth fecundity is a weak predictor of egg size in *B. mori*; both parameters are seemed to be inversely related. In this concern, no data are available in the literature; therefore, the present findings will be discussed on the basis of the results of other lepidopteran species. For example, similar trend has been observed in *Choristoneura* species (Lepidoptera: Tortricidae) where egg size was inversely correlated with rate of egg production (Campbell,1962). However, in the present study, the ANOVA (data of the first part; under publication) indicates that large-egged female moths were significantly highly fecund moths, in the treatment group as compared to their corresponding results in the control group. These results are likely to be due to the added nutritional value of *L. sativum* seed powder in *B. mori* larval feeding diet (mulberry leaves), and probably the sample size used in Pearson correlation analysis was small (n=40) to detect both the type and the strength of the correlation relationship accurately. Also, egg size is reported to be negatively correlated with fecundity in satyrid butterflies (Wiklund and Karlsson, 1984). This suggests that the present *B. mori* larval diet (*i.e.*, mulberry leaves-enriched with *L. sativum* seed powder) is responsible for the production of the remarkable greater numbers of eggs, and also for yielding large-sized eggs; however, at larval feeding on mulberry leaves, without any food additives, there is a tendency for the egg size to be inversely correlated with fecundity. Hence, it seems economically important to pay attention to the shifts in mean values of *B. mori*-egg size that concomitant with the change in the larval diet quality. This speculation consists, to a large extent, with the findings of Campbell (1962), Capinera & Barbosa (1977), and Steinwascher (1982 & 1984).

A weak and marginally non-significant negative correlation (r = -0.27) between *B. mori* egg size and female moth body size, as reflected, respectively, by the egg surface area and the length of the female moth hind femur, has been recorded in the present study. Once again, the ANOVA indicates that the mean values (under publication data) of both *B. mori* egg size and her female moth body size were significantly higher, by *ca*. 23-26% (as measured by the egg surface area) or by *ca*. 44-49% (as measured by the egg volume) and by *ca*. 6-28%, respectively, in the treatment groups than the corresponding mean values in the control groups. Therefore, such a marginal lack of correlation might reveal that the sample size (n = 40) used for Pearson correlation analysis was small to detect this relationship accurately. However, it seems, from the obtained results of the first part (under publication), that *B. mori* larval resources positively affect both female moth body size and fecundity, as well as the egg size; in which large body size may confer a greater advantage to *B. mori* female moths. This speculation might be supported by the data from other insect species which have previously been reported, for example, by Gilbert (1984) who stated that increased body size results in increased fecundity; Johnson (1988 and 1990) who mentioned that the adult body size is positively related to the quantity and quality of food it received as a larva, and the large size in female insects is often associated with an increase in fecundity. Also, Honěk (1993) noted that the adult body size is apparently the most important constraint on the female fecundity. Wiklund and Karlsson (1984) found large butterflies lay large eggs and *vice versa*. Furthermore, Honěk (1993) mentioned that, in general, the slopes of egg size/adult female body size relationships are smaller than those of female fecundity/female size relationships. This is the case in the present study where the slope of egg size/female body size regression was smaller (y = -0.2154x+2.2503) than the slope for fecundity/female size regression (y = -229.82x+1221).

In *B. mori* moths, there was also a weak, but significant, negative correlation (r = -0.35) between fecundity and longevity of moths in the control group; whereas the corresponding relationship in the treatment group was also weak but non-significant and positive (r=0.10). Further, the ANOVA indicates that the averages of female moth longevity in the treatment groups were longer, but not significantly, by *ca*. 2-10% over the average of female longevity in the control group; also, the averages of fecundity were significantly higher, by *ca*. 62–203% in the treatment groups over the corresponding average in the control group (first part data, under publication). Such a non-significant low correlation found between *B. mori* female moth longevity and her fecundity has previously been reported by Miller *et al*. (1982 and 1983) for other silkworms, *viz.,* the giant silkworm moths *Antheraea polyphemus* (Cramer) and *Callosamia promethea* (Drury). Within lepidopteran species, longevity seems to be the most important factor influencing fecundity, and increased fecundity or longevity is correlated with increased longevity or fecundity, in respect (Leather, 1988). Muller *et al.* (2015) reported a similar negative correlation between female longevity and fecundity in the European grapevine moth, *Lobesia botrana*. However, the results of the present study seem to be logical because when *B. mori* female moths investing more in reproduction and depositing a phenomenal number of eggs, on average, *ca*. 958-1256 eggs, in the treatment groups, as compared to about 451 eggs in the control group (under publication data), therefore, these females will have less energy for survival and consequently a negative correlation between these two parameters, fecundity and longevity, has been found. This may suggest another trade-off that might be expected between fecundity (reproduction) and longevity (survival) in *B. mori*. Certainly, the latter suggestion needs a further study. However, Ellers (1996) and Eady *et al.* (2007) have been suggested the existence of a trade-off between fecundity and longevity in the hymenopterous, solitary, larval endoparasitoid, *Asobara tabida* (Nees) and in the bruchid beetle, *Callosobruchus maculatus* (Fabricius), respectively. Furthermore, Jervis *et al*. (2007) noted that the life history trade-off theory suggests that females with a higher fecundity would have shorter longevity; while Milonas *et al*. (2011) found no evidence for a trade-off between fecundity and longevity.

Also, there is a marginally non-significant weak and negative correlation (r = -0.274; *p*=0.087) between *B. mori* female body size (measured as lengths of female moth hind femur) and the total lipid content in *B. mori* female internal reproductive tract at death. Moreover, there is a weak negative or positive, non-significant or marginally non-significant correlation between *B. mori* female moth body size, her fecundity or body weight at death (in the treatment group or in the control group) and the total lipid content in her internal reproductive tract at death (see Tables 2 and 3).

Table 2. Summary of a marginally non–significant correlation between some economic parameters in the mulberry silkworm, *B. mori*

Correlation between	Correlation coefficient (r)			*p*-value			Determination coefficient (r^2)			$F_{(1,38)}$		
	Control	Treatment*		Control	Treatment*		Control	Treatment*		Control	Treatment*	
		c	d		c	d		c	d		c	d
Fecundity and egg volume	– 0.285			0.074			0.082			3.372		
Female moth hind femur length** and egg surface area		– 0.268			0.095			0.072			2.934	
Female moth hind femur length** and total lipid content*** at death	– 0.274			0.087			0.075			3.081		
Female moth hind tibia length** and fecundity			0.283			0.077			0.080			3.312
Female moth forewing length** and fecundity			– 0.269			0.094			0.072			2.958
Male moth hind tibia length** and spermatophore counts per female		0.306			0.055			0.094			3.928	

*c ; d : Continuous and discontinuous larval feeding on mulberry leaves-enriched with *L. sativum* seed powder, respectively.

**As indices of body size.

*** The total lipid content in the internal reproductive tract of *B. mori* female moth at death.

Sample size (n) = 40.

Table 3. Summary for the non–significant (*p*–value ranged from 0.107 to 0.978) correlations between several economic parameters in *B. mori* (Pearson correlation coefficient, r, ranged from + 0.007 to + 0.256 or from – 0.005 to – 0.259)

Correlation between	Correlation direction*			Correlation between	Correlation direction*		
	Control	Treatment**			Control	Treatment**	
		c	d			c	d
Pupal weight and fecundity		+	+	Female forewing length and egg volume	+	–	+
Female moth longevity and fecundity		+	+	Female hind femur length and egg volume	–	–	–
Fecundity and egg surface area		+	+	Female hind tibia length and egg volume	–	+	+
Fecundity and egg volume		+	+	Female forewing length and total protein content	+	+	+
Female moth forewing length and fecundity	–	+		Female hind femur length and total protein content	–	+	–
Female moth hind femur length and fecundity	–	+	+	Female hind tibia length and total protein content	–	+	–
Female moth hind tibia length and fecundity		–		Female forewing length and total lipid content	+	+	+
Spermatophore count and fecundity		–	+	Female hind femur length and total lipid content		–	–
Cocoon shell ratio (%) and fecundity	–	+	+	Female hind tibia length and total lipid content	–	–	–
Male moth forewing length and spermatophore count	–	–	–	Female forewing length and total carbohydrate content	–	–	–
Male moth hind femur length and spermatophore count	+	+	+	Female hind femur length and total carbohydrate content	+	+	+
Male moth hind tibia length and spermatophore count	+		–	Female hind tibia length and total carbohydrate content	–	+	
Female moth forewing length and Female longevity	+	–	+	Fecundity and total protein content	–	+	–
Female moth hind femur length and Female longevity	–	–	–	Fecundity and total lipid content	–	–	+
Female moth hind tibia length and Female longevity	+	+	+	Fecundity and total carbohydrate content	+	+	+
Female moth forewing length and egg surface area	+	–	+	Female body weight at death and total protein content	–	+	+
Female moth hind femur length and egg surface area	–		–	Female body weight at death and total lipid content	+	–	+
Female moth hind tibia length and egg surface area	–	–	+	Female body weight at death and total carbohydrate content	–	+	–

*Positive (+) or negative (–).

**c ; d : Continuous and discontinuous larval feeding on mulberry leaves-enriched with *L. sativum* seed powder, respectively.

Ellers (1996) found similar negative or positive correlation between female fecundity or her body size, in respect, and the fat content of the hymenopterous larval endoparasitoid, *Asobara tabida*. In the meantime, the ANOVA reveals that the mean value of the total lipid content in the internal reproductive tract of *B. mori* female moths at death was, in general, significantly higher by about 2–17%, in the treatment groups, over the corresponding value in the control group (under publication data). Hence, the non-significance or the marginally non-significance here may have an explanation related to the relatively small sample size (n = 40) used to detect accurately such correlation relationships. Another possibility for a trade-off seems to consist of a possible negative correlation (r=-0.27) between *B. mori* female moth body size (as measured by the length of her forewing) and her fecundity (see Table 2). After above, a future experiment should be carried out to demonstrate if *B. mori* female moths which lay greater numbers of eggs (*i.e.,* highly fecund females) do so at the expense of another life-history parameter! On the other hand, regression analysis shows a significant weak negative correlation between spermatophore count per *B. mori* female moth and her fecundity (r = -0.33) in the control group; while in the treatment groups this relationship showed to be non-significant and weakly negative (r = -0.21) or positive (r = 0.07). However, the ANOVA indicates that *B. mori* female fecundity increases (*ca.,* 731.2 – 1364.8 eggs per female on average) with the increasing number of matings (*ca.,* 2.5–3.0 spermatophores per female on average) her partner achieved in the treatment groups as compared to the corresponding mean values in the control group (*ca.,* 450.6 eggs and 1.4– spermatophore per female, in respect; under publication data). Practically, in commercial egg production, the repeated mating performance or the re-use of *B. mori* male moths is common, and four matings can be used without affecting the silkworm economic parameters which relate to egg or silk production (see Jolly *et al.*, 1966; Petkov & Mladenov, 1979; Gupta *et al.,* 1986 and Vijayan *et al.,* 1994). Contradictory results have been reported by Wang (1994) who showed that in order to attain better productivity on the commercial level, the egg or silk production technology should not allow more than two-fold mating; where a many-fold use of *B. mori* male moth for mating, results in decrease in the biological quantitative parameters (Singh *et al*., 2003). However, based on the ANOVA of the first part data (under publication), mating with the same male (repeated matings) is seen to be beneficial and important for reproductive success in *B. mori* female moths. Enhanced-female fecundity which associated with successive or repeated matings has been reported in several insect species (Shorey, 1963; Boggs & Gilbert, 1979; Ridley, 1988; Watanabe, 1988; Oberhauser, 1989; Watanabe & Ando, 1993; Ward & Landolt, 1995; Lamunyon, 1997; Wilson *et al.,* 1999; Arnqvist & Nilsson, 2000; Jiménez-Pérez & Wang, 2004 and Tseng *et al.,* 2007). Also, the ANOVA revealed significant increases in reproductive outputs of both female (*e.g.,* egg number and size) and male moths (*e.g.,* spermatophore counts) in the treatment group; therefore, this finding is more likely due to the following possible explanation: it has been demonstrated that the egg or spermatophore production is a costly physiological event in an insect life cycle (see, for example, Engelmann, 1970; Oberhauser, 1988; Chapman, 1971 and Rivero & West, 2002). Each of this physiologically important product depends on some factors; one of these key factors which affects *B. mori* male or female moth reproductive fitness is the nutritional resources that male or female moths have acquired as larvae (Svärd & Wiklund, 1988; Delisle & Bouchard, 1995; Delisle & Hardy, 1997 and Muller *et al.,* 2015), specifically, *B. mori* moths do not feed. Hence, their reproductive fitness is certainly limited by the nutrients acquired during their larval stage. Therefore, the results of this study could suggest that the mulberry leaves-enriched with *L. sativum* seed powder eaten by *B.mori* male or female moths during their larval stage would positively affect the reproductive performance or fitness of both sexes as noted above.

Additionally, in the treatment group, there was a weak positive, but marginally non-significant, correlation (r=0.306; *p*= 0.055) between the mean body size (as measured by the hind tibia length) of *B. mori* male moth and his mean spermatophore count per female moth. The same trend was noted above with the relationship between the female moth hind tibia length and her fecundity. Such a marginal lack of correlations might be due to a relatively small sample size (n =40) to detect accurately this effect. The present observations might nominate the mean hind tibia length, as an index of *B. mori* moth-body size, to be, somewhat, a good predictor of spermatophore count per female and fecundity in *B. mori* female moths. Also, as *B. mori* male or female moth hind tibia lengths were significantly longer in the treatment group than their corresponding lengths in the control group (first part data, under publication), this would be again due to the enrichment of mulberry leaves with *L. sativum* seed powder during the larval feeding period. So, *L. sativum*-related effect has indicated that the present well–nourished male or female larval diet can positively influence *B. mori* male or female moth reproductive performance *viz.,* spermatophore count, fecundity, egg size, and body size. The latter parameter is known to influence the reproductive success or fitness of both sexes in many insect species (see Campbell, 1962;

Suzuki, 1978; Karlsson & Wickman, 1990; Gupta *et al.*, 1991; Ward & Simmons, 1991; Honěk, 1993; Berg *et al.*, 1997; Gage, 1998; Rivero & West, 2002; Teng & Zhang, 2009 and Muller *et al.*, 2015).

Of the studied 39-correlation analysis, 25-pair of them showed insignificant correlation relationships between *B. mori* parameters present in Table 3. No data are available on *B. mori* to discuss the present findings, with the exception of the results of Mahesha *et al.* (2013) who found a positive correlation (R^2 = 0.506) between *B. mori* fecundity and cocoon shell ratio (%). The observed lack of correlation relationships between the studied parameters in Table 3, in presence or absence of *L. sativum* seed powder, may suggest that in *B. mori* moths, not all the body size indices which noted here are good predictors of the mentioned parameters in Table (3).

CONCLUSION

Pearson correlation analysis which showed a negative correlation between *B. mori* female moth fecundity and the other life-history parameters may shed light on a possible trade-off between fecundity and pupal weight, female longevity, female body size (as reflected by the lengths of hind tibia, or forewing), spermatophore counts per female moth, or egg size (as measured by the egg surface area or volume). Hence, *B. mori* female moths would, then, deposit a great number of eggs at the cost of the above-mentioned life-history parameters. Furthermore, the present study shows *B. mori* pupal weight and the cocoon weight to be important predictors of the cocoon shell weight and the cocoon shell ratio (%), respectively; or in other words, *B. mori* cocoon shell weight or cocoon shell ratio (%) as a function of pupal weight or cocoon weight, respectively, can be predicted from its regression equation.

Therefore, in sericulture field, application of correlation-regression analysis might be advisable either to predict *B. mori* economically important parameters or to encourage for trade-off studies between life-history parameters which are important in *B. mori* rearing or selection programmes.

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AUTHOR CONTRIBUTION

All authors contributed to the study conception and design.

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The authors declare no competing interests.

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الملخص العربي

أهمية عظمى لمسحوق بذور حب الرشاد في صناعة تربية دودة حرير القز

II دراسات ارتباط بين بعض المعايير التكاثرية، المظهرية، البيوكيميائية والإقتصادية لدودة حرير القز

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تمثل الدراسات البحثية الحالية دراسة تكميلية تحت النشر للأهمية الإقتصادية لمسحوق بذور حب الرشاد (*Lepidium sativum*) في صناعة تربية دودة حرير القز. عكست الدراسة الحالية نتائج دراسات ارتباط بين ٣٩ زوج من المعايير التكاثرية، المظهرية، البيوكيميائية والإقتصادية لدودة حرير القز. سجلت الدراسة ارتباط معنوي بين ثمانية أزواج من المعايير موضوع الدراسة وخمسة وعشرون ارتباط غير معنوي، وكما كان هناك ارتباط يمكن الإشارة إليه بالمعنوي لستة أزواج أخرى من المعايير ذات الأهمية الإقتصادية لدودة حرير القز.

أظهرت النتائج أيضاً أن أفضل معيار معنوي للتنبأ بوزن قشرة الشرنقة ونسبة الحرير الناتج من الشرانق هو أوزان كل من العذارى والشرانق–على الترتيب (حيث كان معامل الإرتباط (r) يتراوح– على الترتيب – بين (r = 0.94-0.98, r = 0.55-0.90).

إضافة إلى ذلك فإن علاقات الارتباط المعنوية السالبة التي تم تسجيلها في الدراسة الحالية قد تشير إلى إمكانية وجود تأثير فسيولوجي في اتجاه زيادة عدد البيض الموضوع لكل أنثى وذلك على حساب معايير حياتية أخرى لدودة حرير القز (trade-off) مثل وزن العذارى، طول فترة عمر أنثى فراشة حرير القز، حجم الفراشة، عدد مرات التزاوج أو حجم البيض.

لذا توضح هذه الدراسة مدى الأهمية الإقتصادية لتطبيق التحليل الإحصائي لعلاقات الارتباط والإرتداد بين المعايير المهمة إقتصادياً في مجال صناعة تربية دودة حرير القز وتحسين هذه الصناعة والنهوض بها.