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Biochemical constituents, protein profile and effect of male accessory gland extract on egg production in mother moth of *Antheraea mylitta* Drury

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Abstract: *Male accessory gland factors greatly influence the ovulation and oviposition and male receptivity behaviour of female insects. Some important biochemical constituents available in the male accessory gland extract of Antheraea mylitta during first and second crop grainage were analyzed and injected to just decouple mother tasar moth to observe the impact of extract injection on egg laying parameters. During first crop grainage, in just emerged male moth accessory gland extract the concentration of amino acid was recorded to be 0.049 ± 0.095 to 0.033 ± 0.012 mg/ml, glycerol - 0.007 ± 0.001 to 0.019 ± 0.004 milimole/ml, glycogen 0.827± 0.164 to 1.219 ± 0.060 mg/ml, protein - 9.405 ± 0.417 to 19.305 ± 0.946 to mg/ml, trehalose - 0.080 ± 0.059 to 0.295 ± 0.028 mg/ml and lipid 0.151 ± 0.008 to 0.263±Q.001g/ml. During second crop concentration of amino acid was recorded to be 0.009 ± 0.001 to 0.057±0.002 mg/ml, glycerol - 0.005 ± 0.001 to 0.016±0.0001milimole/ml, glycogen 0.997± 0.700 to 1.482±1.217 mg/ml, protein- 3.389 ± 1.841 to 9.141 ± 3.023 to mg/ml, trehalose - 0.085 ± 0.029 to 0.132 ± 0.036 mg/ml and lipid 0.050 ± 0.012 to 0.510 ± 0.071g/ml. The concentration of total proteins and lipid was also recorded to be in higher proportion than other biochemical constituents. extract of 10µl of single male accessory gland in first crop and 20µl in second crop grainage enhanced fecundity at 26° C. No difference in the qualitative protein profile of male accessory gland extract of 0 to 6 h old males was observed. There were altogether 17 detectable protein bands ranging in between molecular weight of 8.403 to 155.595 kD. When male moths were 28 hr old a higher molecular weight protein band of 184.746kD appeared. Very low molecular protein band of 8.403kD disappeared. When male moths became older i.e of 24 to 36 hours old total 18 bands were seen in the molecular weight range of 155.95 to 8.779kD.*

Key Words: Male accessory glands, Biochemical constituents, Egg laying performance, Temperature

Introduction

The male accessory genital glands of insects may be of ectodermal or mesodermal in origin, known as ectadenia and mesadenia, respectively and this gland consists of a single layer of epithelial cells, the fine structure of which depends on their stage of development and the nature of the secretion produced (Chapman, 1998). The accessory glands become functional in the adult insect and its secretion is involved in several mechanisms linked to reproduction (Landim and Dallacqua, 2005). Mating often induces behavioral and physiological changes in female insects (Yeh and Klowden, 1990). The seminal fluid that is transferred together with sperm can bolster the male's reproductive success in many ways. Seminal fluid proteins cause females to elevate egg laying rate and reduce receptivity towards courting males (Chen, 1984; Chen *et al,* 1988; Kalb *et al,* 1993; Tram and Wolfner, 1999). The chemicals produced by the accessory glands are transferred to the female during copulation,

and they frequently have a long-term effect on her reproductive behavior and physiology (Happ, 1992; Wolfner, 1997; Gillot, 1998, Landim and Dallacqua, 2005). Most of the male reproductive gland secretion constituents are proteins; but smaller molecules, including sugars, lipids (Blum *et al.*, 1967), prostaglandins in Lepidoptera (Gillot, 2003), juvenile hormone in the moths (Shirk *et al.*, 1980) and Diptera (Borovsky *et al.*, 1994) are also present. Secretion also include several peptide and protein hormones as well as enzymes, stress response proteins and immune defence proteins. Large amount of data on the post copulatory stimulation of female reproduction by the male accessory gland in insects is there but no attention has been paid to characterize its secretion (Baer *et al.*, 2001). Nevertheless, postcopulatory stimulation of oogenesis and oviposition is also found in insects (Patricio and Cruz-Landim, 2002). The male accessory glands (MAG) are responsible for the production and secretion of a large number of proteins into the seminal fluid that mix with sperm on ejaculation (Ram and Wolfner, 2007) and these peptide/protein hormones are responsible for a variety of physiological and behavioural responses in the post-mated female, including increased rate of ovulation, loss of receptivity to males, improved sperm storage and increased appetite (Chapman and Davies, 2004; Carvalho *et al.*, 2006). For the maintenance of elevated egg deposition by the females for several days proper storage of sperms is required in female receptecular seminis (Kalb *et al.*, 1993; Neubaum and Wolfner, 1999) and oogenesis, ovulation and egg deposition are part of a multi-step continuous process (Kalb *et al.*, 1993; Xue and Noll, 2000; Heiftz *et al.*, 2010). Male accessory gland components injected into female also act as an antiiaphrodisiac to discourage other males from courting the mated females and seminal fluid can be absorbed by females for use of production of eggs (Pitnick *et al.*, 1997). Thus a wide range of functions have been ascribed to accessory gland products

Tropical tasar silkworm *Antheraea mylitta* Drury is a wild sericigenous silkworm which produces lustrous tasar silk. Tasar culture is mainly practiced in tropical India. The Daba bivoltine ecorace of tasar silkworm is mainly reared in two seasons. A seed crop which is raised during July- August followed by a commercial crop during September to November. The part of the seed cocoons of second crop are preserved till next June Male and female moths emerge from the diapausing pupae. The distribution of this species is in the varied range of agro-climatic conditions (Mishra *et al.*, 2010). During grainage the problem of low fecundity is often seen leading to less production and productivity. As MAG products are reported to enhance fecundity, an attempt has been made in the present study to analyze some important biochemical constituents in the male accessory gland extract in the males of both first and second crop of different age. Newly emerged female moths are kept in coupled state for six to eight hours. Thereafter the mother moths are decoupled and kept for egg laying for 72 hours. In the present study, an attempt has been made to see the effect of injection of a single male accessory gland extract on different egg laying parameters of mother tasar moth. Simultaneously, different biochemical constituents have also been analyzed in the extract in both the crops. The protein profile of male accessory gland extracts has also been studied in different aged male moths.

Materials and Methods

The male moths of *A. mylitta* were collected in both I and II crop grainage. The MAG was dissected out in the cold silkworm saline (pH 7.0), weighed and by weight it was 50 mg. Thus weight MAG extract was prepared by crushing it in 500µl of silkworm saline with a Polytron homogenizer thus a 10% (wt./volume) solution

was prepared. The crushed material was centrifuged at 5000 rpm at 4°C and the supernatant was used for the experimental study. The extract of a single MAG was analyzed for the presence of quantitative protein after, amino acids , glycogen , trehalose and total lipid. The quantum of extract injected was 5, 10, 20, 50 and 100 µl in different batches of 100 mother moths in three replications. A separate control lot was also kept where mother moths were only silkworm saline was injected. Thereafter the mother moths were kept for egg laying for 72 hours. After 72 hours, all the mother moths were dissected out and the number of un-laid and undeveloped eggs was counted. Based on the laid and un-laid eggs the co-efficient of egg laying was calculated by following formula:

$$\text{Coefficient of egg laying} = \frac{\text{Number of eggs laid}}{\text{Total number of laidand un-laid eggs}} \times 100$$

The estimation of quantitative protein was done following the method of Lowry *et al.,* (1951) and that of amino acids after Moore and Stein, (1948). The estimation of quantitative trehalose and glycogen was done following the method of Wyatt and Kalf, (1957). An enzymatic method for the estimation of glycerol in haemolymph was followed after Hagen and Hagen, (1962) and the estimation of lipid was after Folch *et al.,* (1957). Qualitative presence of the male accessory gland extract protein profile of 0, 6, 12, 18, 24 and 36 h old male moths was studied through one dimensional Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) following the method of Laemmli (1970). The important lanes where difference was seen in the qualitative protein profile was further analyzed with the help of Gel Electrophoresis. Healthcare software used was IMAGEQUANTTL for densitometry and exact molecular weight of proteins. Thus recorded data on biochemical constituents were subjected to t-test to record the difference between two crops and data on different egg laying parameters like laid, un-laid, undeveloped, total eggs and co-efficient of egg laying percent (CE%) were subjected to statistical analysis with three way ANOVA so as to find out significant and non-significant difference with respect to temperature and quantum of extract injected into just decoupled females following the method of Panse and Sukhatme (1985).

Results and Discussion

During first crop grainage, in just emerged male moth accessory gland the concentration of amino acid was recorded to be 0.049 ± 0.095 to 0.033 ± 0.012 mg/ml, glycerol - 0.007 ± 0.001 to 0.019 ± 0.004 milimole/ml, glycogen 0.827± 0.164 to 1.219 ± 0.060 mg/ml, protein - 9.405 ± 0.417 to 19.305 ± 0.946 to mg/ml, trehalose - 0.080 ± 0.059 to 0.295 ± 0.028 mg/ml and lipid 0.151 ± 0.008 to 0.263±0.001g/ml. The concentration of total proteins and lipid was recorded to be in higher proportion than other biochemical constituents. With the increase of age a fluctuation in the concentration of these constituents was observed (Table 1, 2 and 3; Fig. 2 a, b, c, d, e, f).

In the male accessory gland extract of second crop concentration of amino acid was recorded to be 0.009 ± 0.001 to 0.057±0.002 mg/ml, glycerol - 0.005 ± 0.001 to 0.016 ± 0.0001 milimole/ml, glycogen 0.997± 0.700 to 1.482±1.217 mg/ml, protein- 3.389 ± 1.841 to 9.141 ± 3.023 to mg/ml, trehalose - 0.085 ± 0.029 to 0.132 ± 0.036 mg/ml and lipid 0.050 ± 0.012 to 0.510 ± 0.071g/ml. The concentration of total proteins and lipid was also recorded to be in higher proportion than other biochemical constituents. The fluctuation in the concentration of these constituents was observed in older male (Table 1, 2 and 3; Fig. 2 a, b, c, d, e, f). The concentration of biochemical constituents was significantly higher ($P < 0.01$) in most of the cases during first crop than second crop male in moth accessory gland extract.

Table 1. Total amino acids and glycerol in the male accessory gland extract of *A. mylitta* in first and second crop

Age of male moth (h)	Amino acids (mg/ml)		t Stat	Glycerol (milimole/ml)		t Value
	First Crop	Second Crop		First Crop	Second Crop	
0	0.049±0.001	0.025±0.002	117.793**	0.007±0.0001	0.005±0.001	12.945**
6	0.043±0.001	0.025±0.002	60.572**	0.008±0.000	0.006±0.001	8.725**
12	0.025±0.013	0.023±0.002	NS	0.009±0.001	0.006±0.001	8.294**
18	0.021±0.0001	0.009±0.001	62.029**	0.011±0.002	0.007±0.008	7.112**
24	0.071±0.022	0.010±0.001	8.353**	0.011±0.003	0.007±0.001	3.711**
30	0.095±0.027	0.021±.001	8.280**	0.019±0.004	0.008±0.001	7.634**
36	0.095±0.027	0.040±0.002	5.994**	0.019±0.004	0.009±0.001	6.723**
48	0.033±0.012	0.057±0.002	5.141**	0.007±0.000	0.016±0.0001	35.230**

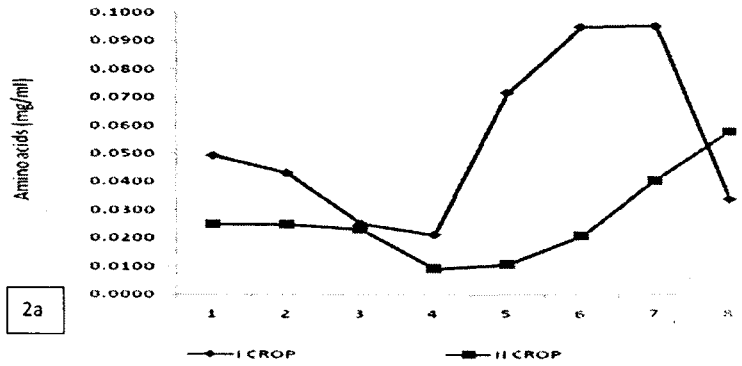
Table 2. Total glycogen and protein in the male accessory gland extract of *A. mylitta* in first and second crop

Age of male moth (h)	Glycogen (mg/ml)		t Stat	Protein (mg/ml)		t Stat
	First Crop	Second Crop		First Crop	Second Crop	
0	1.219±0.060	1.015±1.008	9.693**	10.926±1.723	6.018±2.453	8.451**
6	0.839±0.033	1.001±1.001	7.939**	9.405±0.417	5.726±2.393	26.418**
12	0.933±0.185	0.997±0.700	NS	15.873±1.449	7.779±2.789	15.741**
18	1.007±0.041	1.029±1.014	NS	6.029±2.127	5.111±2.261	1.292*
24	1.096±0.135	0.979±0.599	2.508*	13.872±3.621	3.389±1.841	8.683*
30	1.320±0.199	1.075±1.037	3.622*	13.927±1.972	9.141±3.023	6.490*
36	1.320±1.149	1.098±1.048	2.642*	13.927±0.199	8.698±1.048	7.944*
48	0.827±0.164	1.482±1.217	11.579**	19.305±0.946	6.341±2.518	40.770**

Table 3. Total trehalose and lipid in the male accessory gland extract of *A. mylitta* in first and second crop

Age of male moth (h)	Trehalose (mg/ml)		t Stat	Lipid (g/ml)		t Stat
	First Crop	Second Crop		First Crop	Second Crop	
0	0.080±0.059	0.084±0.005	NS	0.210±0.009	0.050±0.012	32.044**
6	0.151±0.018	0.114±0.338	2.604	0.263±0.001	0.142±0.038	17.094**
12	0.138±0.035	0.122±0.103	NS	0.182±0.007	0.237±0.049	7.809*8
18	0.199±0.022	0.132±0.036	4.230	0.147±0.012	0.510±0.071	18.034**
24	0.284±0.028	0.177±0.042	6.135	0.151±0.008	0.492±0.170	16.337**
30	0.143±0.021	0.139±0.085	NS	0.214±0.009	0.576±0.276	51.841**
36	0.295±0.021	0.085±0.029	7.914	0.214±0.146	0.109±0.033	23.041**
48	0.128±0.023	0.111±0.333	NS	0.226±0.002	0.123±0.035	15.414**

The performance of egg laying during first crop grainage by a single mother moth injected with 5, 10, 20, 50 and 100µl of a single MAG of different concentration of 10% (wt./volume) and control only with same quantity of silkworm saline and kept for egg laying at 30, 26 and 22°C temperature is shown in Fig. 3 a and b and Table - 4 during first crop and Fig. 4 a and b and Table - 5. It may be seen from Table 3 that the egg laying performance was recorded to be highest when mother moths were injected with 10µl of 10% MAG extract at 26°C. The number of eggs laid was recorded to be highest *i. e.*, 277 and co-efficient of egg laying was also


Amino acids (mg/ml)
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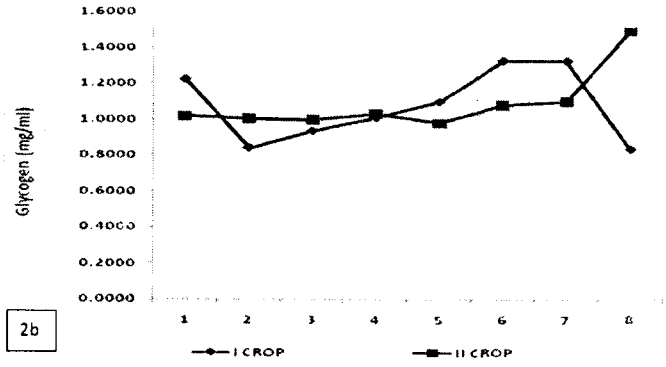
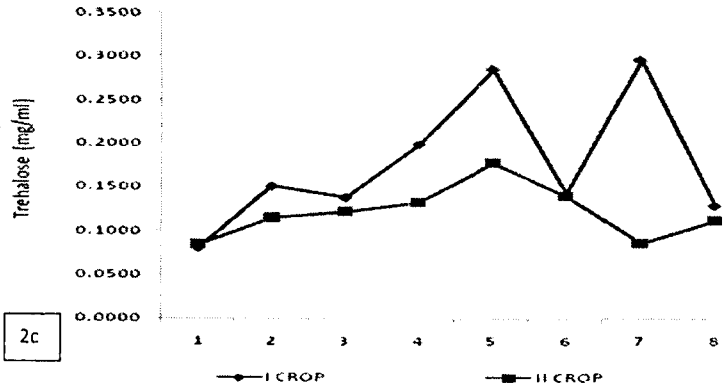
0.0900
0.0800
0.0700
0.0600
0.0500
0.0400
0.0300
0.0200
0.0100
0.0000
1 2 3 4 5 6 7 8
I CROP
II CROP

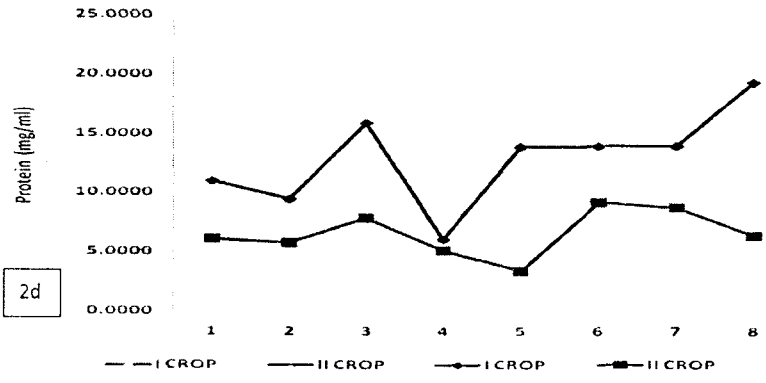
2a


Glycogen (mg/ml)
1.6000
1.4000
1.2000
1.0000
0.8000
0.6000
0.4000
0.2000
0.0000
1 2 3 4 5 6 7 8
I CROP
II CROP

2b


Trehalose (mg/ml)
0.3500
0.3000
0.2500
0.2000
0.1500
0.1000
0.0500
0.0000
1 2 3 4 5 6 7 8
I CROP
II CROP

2c


Protein (mg/ml)
25.0000
20.0000
15.0000
10.0000
5.0000
0.0000
1 2 3 4 5 6 7 8
I CROP
II CROP
I CROP
II CROP

2d

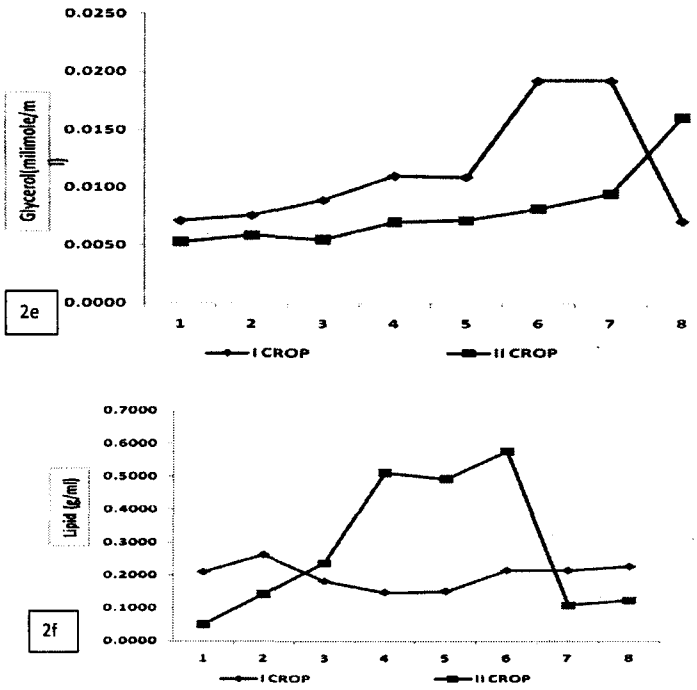
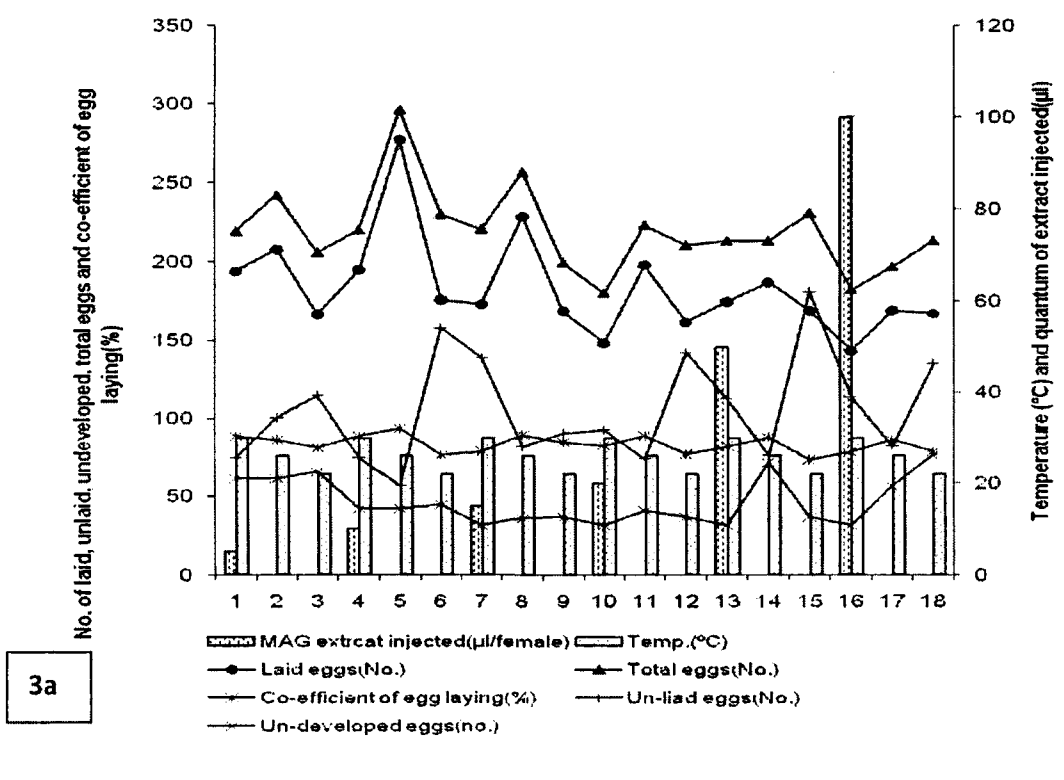


Fig. 2 a, b, c, d, e and f Different biochemical constituents in male accessory gland extract of *A. myliita* I and II crop



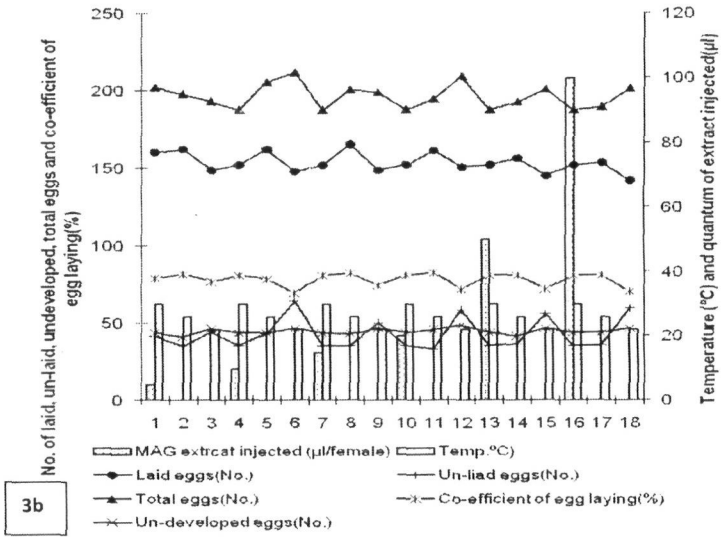


Fig. 3 Egg laying performance of mother tasar moth after injection of different concentration of male accessory gland extracts (A) *vis a vis* control (only silkworm saline- B) during first crop.
Note: Values on X Axis: 1-3- 5µl; 4 - 6: 10µl; 7-9: 15µl; 10-12: 20µl;13-15 µl and 16-18:100 µl of extract or silkworm saline injected and moths kept at 30, 26 and 22°C for recording egg laying parameters.

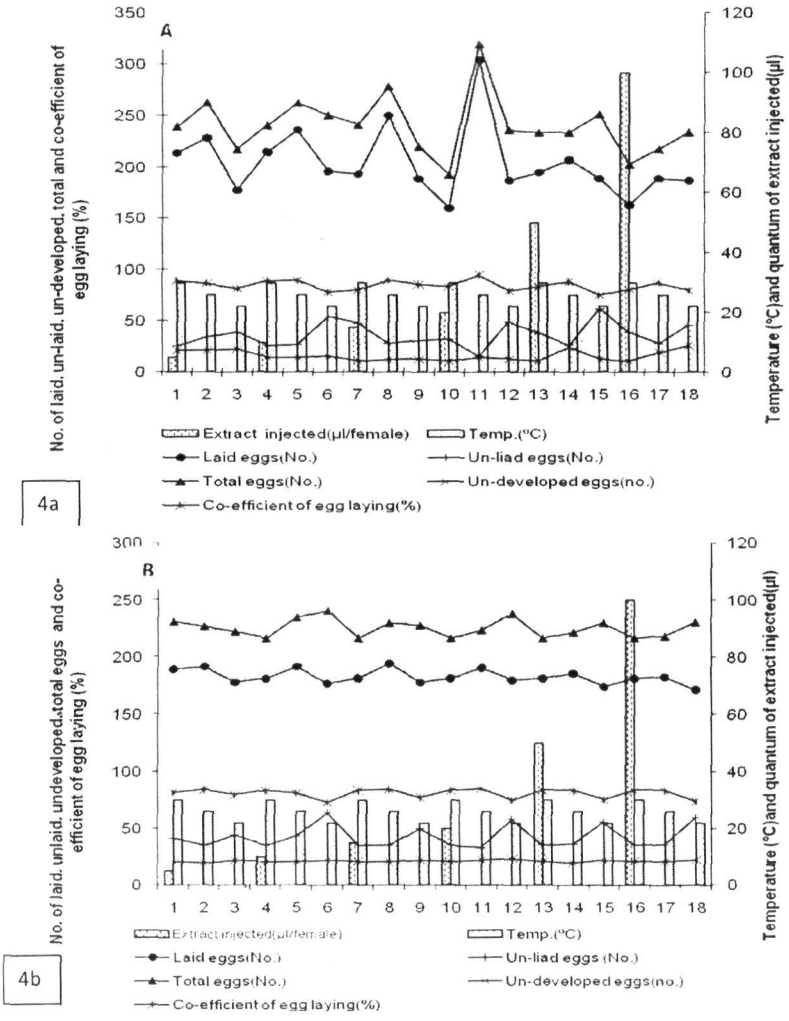


Fig. 4 Egg laying performance of mother tasar moth after injection of different concentration of male accessory gland extracts (A) *vis a vis* control (only silkworm saline - B) during second crop.
Note: Values at X Axis: 1-3- 5µl; 4 - 6: 10µl; 7-9: 15µl; 10-12: 20µl;13-15 µl and 16-18:100 µl of extract or silkworm saline injected and moths kept at 30, 26 and 22°C for recording egg laying parameters.

Table 4. Egg laying parameters of mother moth of *A. mylitta* during first crop

Treated (MAG in extract)	MAG extract injected (µl/female)	Temp. (°C)	Laid eggs (No.)	Unlaid eggs (No.)	Total eggs (No.)	Un-developed eggs (no.)	Co-efficient of egg laying (%)
	5	30	194	25	219	21	88.42
		26	208	34	242	21	85.80
		22	167	39	206	22	80.92
	10	30	195	26	220	14	88.43
		26	277	19	296	14	93.45
		22	176	54	230	15	76.49
	15	30	173	48	221	11	78.43
		26	229	28	257	12	89.07
		22	169	31	200	13	84.53
	20	30	149	32	180	11	82.41
		26	198	25	223	14	88.68
		22	162	49	211	13	76.83
	50	30	175	39	213	11	81.96
		26	187	26	213	25	87.76
		22	169	62	231	13	73.15
	100	30	144	39	183	11	78.68
		26	169	28	197	19	85.74
		22	167	46	213	26	78.30
Control (only silkworm saline)	5	30	160	42	202	21	79.41
		26	162	35	198	20	82.10
		22	149	45	193	22	76.97
	10	30	152	36	188	21	81.08
		26	162	43	206	21	78.91
		22	148	64	212	22	69.74
	15	30	152	36	188	21	81.08
		26	165	35	201	21	82.37
		22	149	50	199	22	74.72
	20	30	152	36	188	21	81.08
		26	161	33	195	22	82.85
		22	151	59	209	23	72.04
	50	30	152	36	188	21	81.08
		26	156	36	193	20	81.12
		22	145	56	201	22	72.18
	100	30	152	36	188	21	81.08
		26	153	36	189	21	81.04
		22	142	60	202	22	70.47
CD at 5%							
Treatments (TR.)			2.71	2.86	4.09	1.58	0.92
Concentration (CONC.)			4.69	4.96	7.08	2.74	1.59
Temperature (TEMP.)			3.32	3.51	5.01	1.94	1.12
TR. x CONC.			6.63	7.01	10.02	3.87	2.24
TR. X TEMP.			4.69	4.96	7.08	2.74	1.59
CONC. x TEMP.			8.12	8.59	12.27	4.74	2.75
TR. x CONC. x TEMP.			11.49	12.15	17.35	6.71	3.89

Table 5. Egg laying parameters of mother moth of *A. mylitta* during second crop

Treated (MAG extract)	Extract injected (µl/female)	Temp. (°C)	Laid eggs(No.)	Un-laid eggs (No.)	Total eggs(No.)	Un-developed eggs(no.)	Co-efficient of egg laying (%)
	5	30	214	25	239	21	89.39
		26	228	34	262	21	86.89
		22	178	39	217	22	81.89
	10	30	215	26	240	14	89.39
		26	236	26	262	14	89.94
		22	196	54	250	15	78.37
	15	30	193	48	241	11	80.22
		26	250	28	278	12	89.89
		22	189	31	220	13	85.94
	20	30	161	32	192	11	83.51
		26	304	15	319	14	95.21
		22	187	49	236	13	79.29
	50	30	195	39	233	11	83.50
		26	207	26	233	25	88.81
		22	189	62	251	13	75.29
	100	30	164	39	203	11	80.79
		26	189	28	217	19	87.05
		22	187	46	233	26	80.16
Control (Only silkworm saline)	5	30	189	42	231	21	81.99
		26	191	35	227	20	84.39
		22	178	45	222	22	79.97
	10	30	181	36	217	21	83.61
		26	191	43	235	21	81.52
		22	177	64	241	22	73.38
	15	30	181	36	217	21	83.61
		26	194	35	230	21	84.60
		22	178	50	228	22	77.94
	20	30	181	36	217	21	83.61
		26	190	33	224	22	85.08
		22	180	59	238	23	75.44
	50	30	181	36	217	21	83.61
		26	185	36	222	20	83.59
		22	174	56	230	22	75.69
	100	30	181	36	217	21	83.61
		26	182	36	218	21	83.55
		22	171	60	231	22	74.19
CD at 5%							
Treatments (TR.)			13.16	3.05	13.07	1.58	1.07
Concentration (CONC.)			22.80	5.29	22.64	2.74	1.86
Temperature (TEMP.)			16.12	3.74	16.01	1.94	1.32
TR. x CONC.			32.24	7.48	32.02	3.87	2.63
TR. x TEMP.			22.80	5.29	22.64	2.74	1.86
CONC. x TEMP.			39.49	9.16	39.22	4.74	3.22
TR. x CONC. x TEMP.			55.85	12.96	55.46	6.71	4.56

recorded to be maximum (93.45%). The moths injected with higher quantity of MAG extract laid lesser number of eggs, such moths also retained more number of un-laid eggs and undeveloped eggs. The moths kept at 30 °C laid lesser number of eggs. At low temperature of 22°C more undeveloped eggs were present in the abdomen of mother moths. The mother moths only injected with silkworm saline laid less number of eggs with increased volume of silkworm saline.

Similar results were also observed during second crop grainage but with a difference that the quantum of single MAG extract required to get highest number of laid eggs (304) was 20µl with highest co-efficient egg laying percent of 95.21% at 26°C (Fig. 3 a and b and Table – 5). This possibly may be due to comparatively lesser quantity of biochemical constituents available in the extract of MAG in second crop than first crop.

Qualitative protein profile of male accessory gland extract is shown in Fig.5. There was no difference in the protein profile of male accessory gland extract of 0 to 6 h old males. There were altogether 17 detectable protein

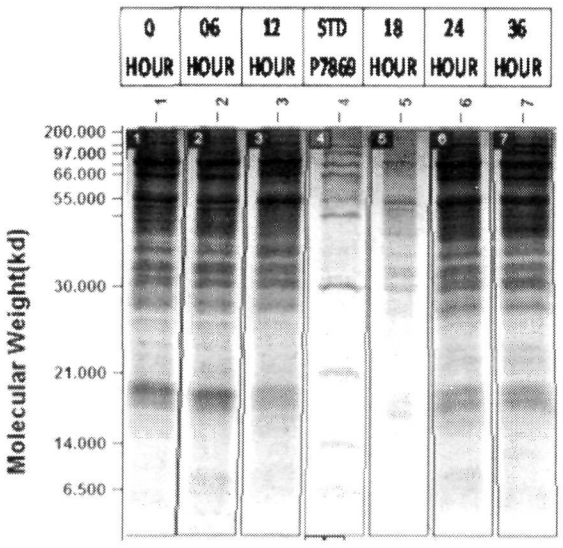


Fig.5 Accessory gland extracts protein of *A. myliita* among different age (h) male moths

bands ranging in between molecular weight of 8.403 to 155.595 kD (Fig.6a). When male moths were 28 h old a higher molecular weight protein band of 184.746kD and total 19 bands in a molecular weight range of 17.316 to 184.746 kD were detected. Very low molecular protein band of 8.403kD disappeared (Fig.6b). When male moths were 24 to 36 hours old total 18 bands were seen in the molecular weight range of 155.95 to 8.779kD (Fig.6c). A comparative analysis of the sigma make protein standards in molecular weight range of 200 to 6.5kD is shown in Fig.6d.

Normal reproduction in the insects is a physiological syndrome with nutritional and neuro-endocrine interactions and ecological implications (Engelman, 1970; Calow, 1973). Oviposition is one of the most important steps in the process of sexual reproduction. For the successful oviposition of eggs by a female moth, several factors and events play important roles, these include nutritional, environmental, hormonal, chemical and behavioural (Yamaoka and Hirao, 1977, 1981; Katti *et al.,* 2007). It is estimated that about 2.95% of the total assimilated food by a female *A. mylitta* is diverted towards egg production (Rath *et al.,* 2003). The optimum mating duration is essential for insemination of sufficient sperms to get more fertility in *A. mylitta* (Rath *et al.,* 2002).

A part from above observations of the various authors the role of MAG factors/substances fecundity enhancement have also been demonstrated in many experimental designs starting with accessory gland implantation or injection of crude extract of gland secretion into females (Friedel and Gillot, 1976; Morrison *et al.,* 1982; Chen *et al.,* 1988). Male accessory gland derived factors can stimulate oogenesis and enhance oviposition in insects (Jin and Gong, 2001). Seminal fluid proteins and sperms, both are required to stimulate oogenic progression and egg deposition in insects (Heifetz *et al,*

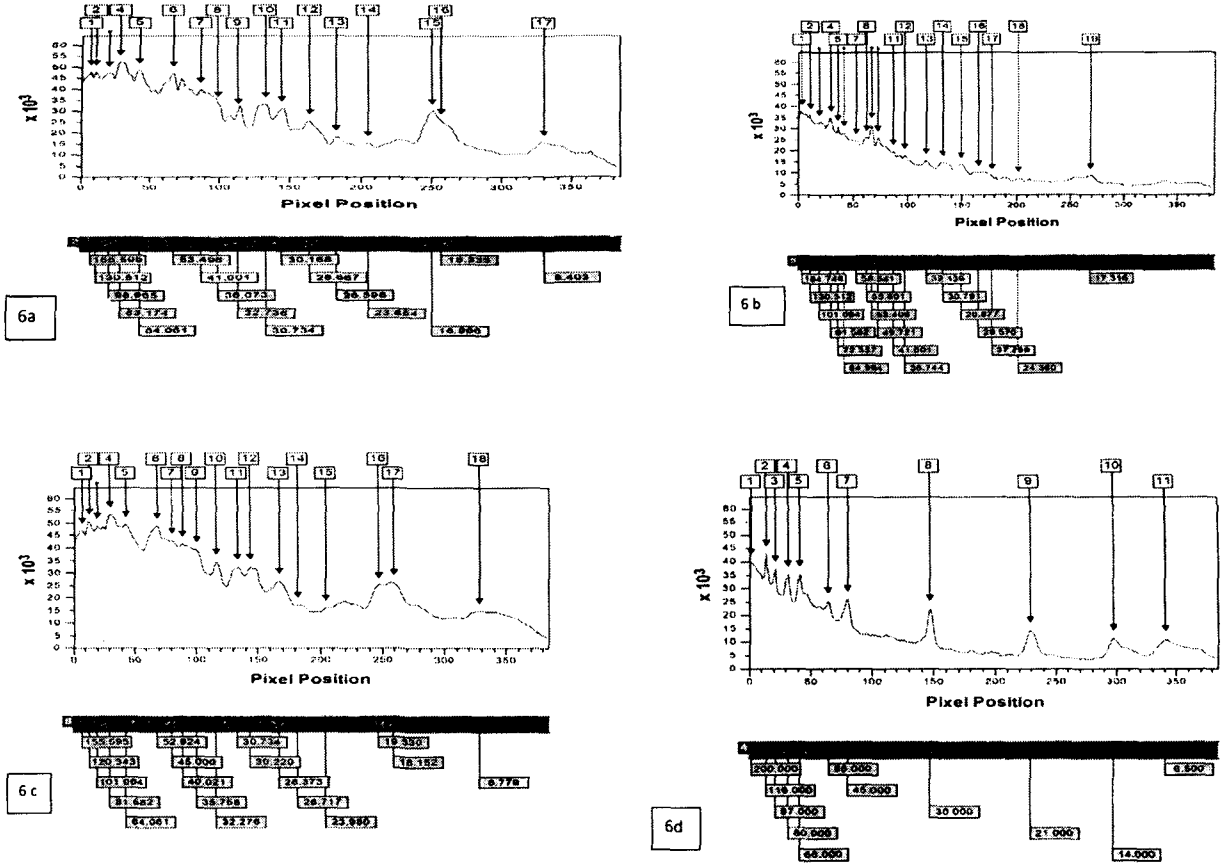


Fig. 6 Presence of different protein bands in the male accessory gland extract vis-à-vis control (6a:in 6 h old male, 6b:18 h old male,6c:24 h old male and 6d: standard

2010). In *D. melanogaster*, the male accessory glands (AG) are responsible for the production and secretion of a large number of proteins into the seminal fluid that mix with sperm on ejaculation (Ram and Wolfner, 2007). These include several peptide and protein hormones as well as enzymes, stress response proteins and immune defense proteins. The peptide/ protein hormones are responsible for a variety of physiological and behavioral responses in the post-mated female, including increased rate of ovulation, loss of receptivity to males, improved sperm storage and increased appetite (Carvalho *et al.*, 2006; Chapman and Davies, 2004). In *Pyrrhocoris apterus* the amounts of the total proteins and 53 kDa protein in male accessory glands of the firebug increased with age of the adult life. The 53 kDa protein, the most abundant polypeptide detected in the secretion of the AGs, and some other smaller peptides were identified as glycoproteins (Socha *et al.*, 2004).

A detailed analysis of the spermathecal fluid proteins indicated that they fall into a range of different functional groups, most notably enzymes of energy metabolism and antioxidant defense and also facilitates long term storage of sperm in female receptecular seminis (Baer *et al.*, 2009). Sperm storage by females is widespread throughout the animal kingdom (Birkhead and Moller, 1998) and females also provide specialized morphological structures for sperm storage often known as spermathecae (Eberhard, 1996). The secretions of male accessory glands contain

proteins, metabolites and other chemicals in the honeybee *Apis mellifera* (Klenk *et al*, 2004). Spermathecal fluid has recently been shown to maintain sperm viability (den Boer *et al.*, 2009) . Several proteins have been proposed to be responsible for this effect, such as the glycolytic enzyme triosphosphate isomerase (Klenk *et al*, 2004) and a number of antioxidant defense enzymes (Collins *et al.*, 2004). Males transfer a complex mixture of components to the female along with sperm (Tozetto *et al.*, 2007; Findlay *et al.*, 2008). In total, eleven peptidases including aminopeptidases, endopeptidases and a γ-glutamyl transpeptidase have already been identified as AG products in *D. melanogaster* (Mueller *et al.*, 2004; Walker *et al.*, 2006). One of these peptidases, an astacin-like endopeptidase, is involved in the cleavage of the male AG ovulin in the female reproductive tract to produce four products, two of which stimulate ovulation in the first 24 h post-mating (Ravi Ram *et al.*, 2006).

In recent years the different MAG specific proteins (Acps) have been characterized and their role in female moth receptivity, increased rate of egg laying, and protection of sperms in the receptecular seminis of mother moths have been examplified (Heifetz *et al*, 2010). In the present study too the role of MAG factors is proved to be crucial in enhancing egg laying efficiency of *A. mylitta* mother moth and is conformity with the findings of these authors. A temperature of 26°C is required to have maximum egg laying performance. A single male accessory gland of 10µl and 20 µl of extract injected into the abdomen of mother moth of *A. mylitta* enhances fecundity during I and II crop grainages, respectively. However, presence of different bands of proteins and other fecundity enhancing substances present in the male moth accessory gland of *A. mylitta* are needed to further characterize with their physiological significance for its better utilization by the tasar silk industry.

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