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THE VARIABILITY OF CHITIN DEPOSITION IN THE BODY WALL/ INTEGUMENT OF FIFTH INSTAR LARVAE OF SILK WORM, *BOMBYX MORI* (L) (RACE: PM X CSR_2) RECIPIENT OF ACETONE EXTRACTIVES OF SOME NON MULBERRY PLANTS

Sharad G. Jagtap
Research Fellow (Reg.No.15112216) Shri Jagdishprasad Jhabarmal Tibrewala University
(K. G. Kataria College, Daund, Pune-413801)

ABSTRACT
Phytophagus insects, like silkworm, *Bombyx mori*_(L), derive nutrients & growth promoting biocompounds from the variable or specific flora available for them. The plants are the richest source of juvenile hormone analogues for phytophagus insects like silkworm, *Bombyx mori* (L). Fraction of plant derived insects juvenoids serve to take pause in the progression of metamorphosis through arresting some of the biochemical reactions including chitin synthesis or accelerating progression through other biochemical pathways in the larval body of insects. Ten microliters of each selected concentrations of acetone extractives of selected non mulberry flora (*Vitis vinifera; Alstonia scholaris; Santalum album; Lantena camera; Syzyguim cumini and Tectona grandis*) and acetone solution of camphene were topically applied to individual larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR_2) at 48 hours after the fourth moult .Body wall chitin of untreated control larvae ; acetone treated control larvae & treated larvae was estimated at zero; 24; 48; 72; 96 and 120 hours after the fourth moult. Body wall chitin (quantity and amount of deposition) in the untreated control group of silkworm larvae at zero; 24; 48;72;96 and 120 hours after the moult was found measured as : 6.841 (+0.172),0.000; 7.114(+0.786),0.273; 9.512(+0.793),2.671; 17.849 (+1.123),11.008; 19.812(+1.437),12.971 and 23.736(+4.158),16.895 units respectively. Topical application of selected concentrations of acetone extractives of selected non mulberry plants and camphene to fifth instars larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR_2) was found effected into the reduction in the deposition of chitin in the larval body wall. This reduction in body wall chitin was found ranging from zero to ninety nine percent. The plot of concentrations of acetone extractives of plant and percent reduction in the body wall chitin was found exhibiting a characteristic S- form of displacement, which herewith titled as Punyamayee Dose Response Curve. Since the effects of juvenoids involve the inhibition of metamorphosis through reduction in chitin deposition, it is possible to express the concentration (dose) applied in terms of ID_{50} value. The ID_{50} value of juvenoid contents of selected non mulberry flora can be defined as the specific unit (microgram), which enable chitin to deposit fifty percent (only) in the body wall of larvae (In comparison with untreated control). Accordingly ,the ID_{50} values calculated from the Punyamayee Dose Response Curves for non mulberry plants : *Vitis vinifera; Alstonia scholaris; Santalum album; Lantana camera; Syzyguim cumini; Tectona grandis* and Camphene solution were found measured 1.27; 1.40; 2.325; 2.86;3.60; 4.04 and 1.00 micrograms respectively. The variation in the ID_{50} values among the non mulberry flora for the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR_2) in the study may be concerned with quantity of acetone soluble juvenoid contents of the plants. Acetone soluble juvenoid content of non mulberry flora may be utilized efficiently for the fortified development of fifth instars of silkworm, *Bombyx mori* (L) & thereby, the cocoon quality. Sigmoid (S-form) Punyamayee Dose Response Curve may help for quantitative estimation of juvenoid contents of various plants and Camphene like synthetic compounds.

KEY WORDS: Juvenile Hormone Analogues, Silkworm, *Bombyx mori.*

INTRODUCTION

The concentration of juvenile hormone (JH) & moulting hormone (MH) serve to orchestrate the progression of metamorphosis in the insects. The principal function of juvenile hormone (JH) in insects like silkworm, *Bombyx mori* (L). The JH is the secretary product of specialized endocrine glands, the corpora allata present in the cephalic region of insects is selective inhibition of morphogenetic program at predetermined and group specific ontogenic positions (Zaoral & Slama, 1970). The term juvenoid was proposed for compounds (Plant derived; animal derived & synthetic) that are exhibiting biochemical properties of JH in the insects (Williams, 1956). Natural product with JH activity that have been isolated from animals & plants represent a rather small but important fraction when compared to synthetic juvenoids like Farnasol Methyl Ether (FME), methoprene, hydroprene, isoprene, kinopren etc. Plants are the richest source of natural juvenoid for phytophagous insects. Exogenous topical application of plant material through suitable solvent exhibited potent activity through massive turnover, alteration of constituency of metabolites like proteins, lipids, carbohydrates, aminoacids, fatty acids & chitin too (Gopakumar *et al*., 1977; Slama, 1979; Khyade *et al*., 2002; Khyade *et al*., 2003 & Khyade, 2004). Juvenile hormone (JH) and Juvenile Hormone analogues(JHA) or juvenoids are well known to prolong the larval life; improve the physiological status of larval body of insects

and therefore, they have been tried for qualitative improvement of silk (Grenier & Grenier, 1983; Kamimura & Kiuchi, 1988; Ratnasen, 1988; Mamatha *et al*., 1999 & Khyade , 2002, 2003 & 2004). Juvenomimetic activity through acetone extractives was observed by Gopakumar, *et al* (1977) in the South Indian Flora & imagined the probability of juvenomimetic action in other plants. The larvae of insects especially phytophagus, manage for interplay of natural juvenile hormone from their body, juvenoid contents from plants (& moulting hormone too) & allow to orchestrate the progression from one instar / stage to the next. The moulting hormone/ ecdyosteriod serves for regulating the onset & timing of moulting cycle. The juvenile hormone & juvenoids regulate the quality of the moult (Riddiford, 1985, 1994 & Signal, 1985). During the last stadium of holometabolous insects like silkworm, *Bombyx mori* (L), reduction in the titer of juvenile hormone (JH) in haemolymph is necessary step in the initiation & metamorphosis (Calvez, *et al.,* 1976). Bioassay of juvenile hormone activity (Juvenoid) has been amongst exclusively based on the evaluation of heterochronic deviations caused in insect metamorphosis. The favourite objects of evaluation of juvenoid effects have always been partly adult mosaic intermediates generally known as adultoids (Slama, 1985). Since the effects of juvenoids mostly involve inhibition of metamorphosis through change in the rate of biochemical reactions including the chitin deposition, it become easier to express the content ration (dose) of juvenoid content, topically applied in specific terms (units). According to Slama (1974), the juvenoid activity may be expressed in terms of unit, which deposit known percent less chitin in the body wall of insect larvae. Concentration (dose) of juvenoid, topically applied to sensitive stages of insect development, at the specific period may be concerned with percent reduction in chitin deposition in the larval body organs like integument(Vitthalrao B. Khyade, 2006). With the supposition, the efforts were carried out to screen acetone extractives of selected non mulberry flora for juvenoid action in silkworm, *Bombyx mori* (L).

TABLE-1 : Plants with insect Juvenoid activity

Sr. No.	Plant	Juvenoid Content (FME)
1	Acalypha hispida (L)	61
2	Anacardium occidentale (L)	20
3	Bauhinia accuminata (L)	61
4	Bougainvilla glabra (L)	31
5	Cocos nucifera (L)	61
6	Hibiscus rosasinesis (L)	20
7	* Lantana camera (L)	132
8	Malvaviscus Populinus (L)	31
9	Morinda tinctoria (L)	61
10	Morus alba (L)	102
11	Nycanthus arbor (L)	61
12	Pterocarpus marsupium (L)	82
13	*Santalum album (L)	184
14	* Tectona grandis (L)	128
15	Terminalia paniculata (L)	60
16	Verteria indica (L)	44
17	*Vitis vinifera (L)	367
18	Sesamum indicum (L)	92

* - Selected for present study.

Source : 1. Current Science, 42, Np.20: 725-726.
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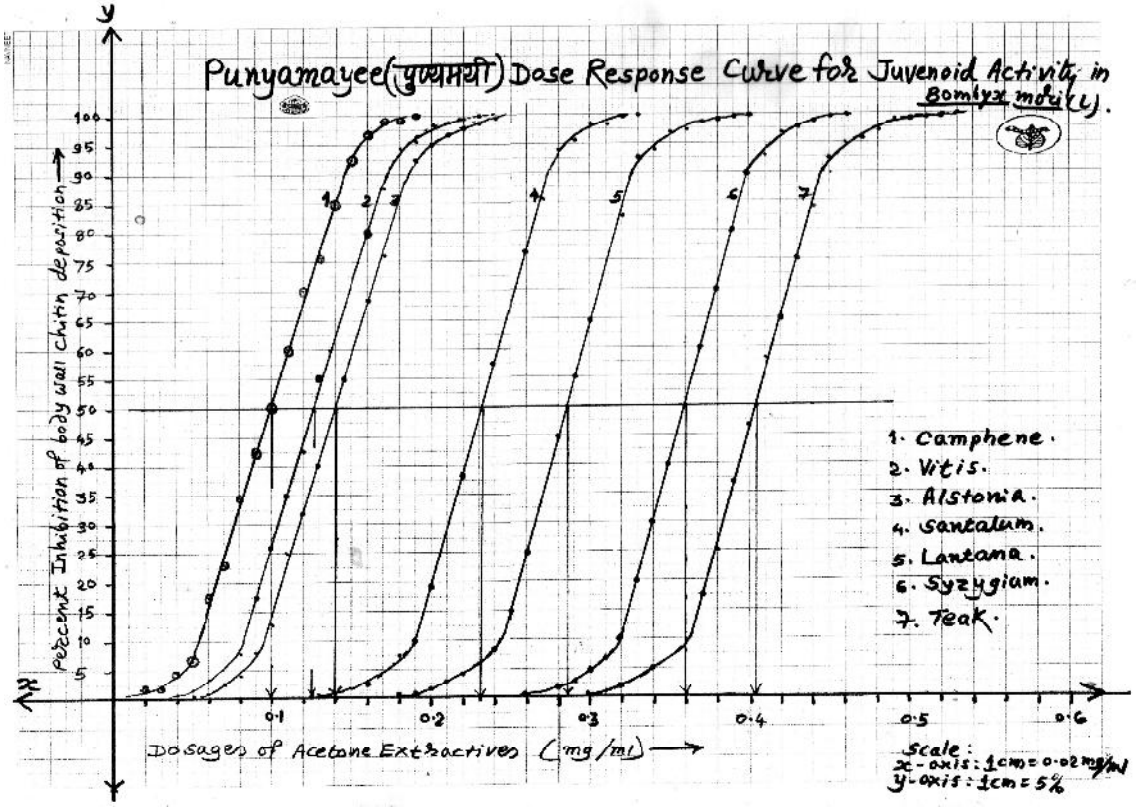
MATERIAL AND METHODS

The material and methods have been divided into the parts like: Collection of non-mulberry plants; shade drying; extraction, topical application and chitin bioassay. Based on the availability and intensity of juvenoid content, the non mulberry plants from the list, in table: 1 were selected for screening and they include : *Vitis vinifera* (L); *Santalum album* (L); *Lantana camera* (L) and *Tectona grandis* (L). *Alstonia scholaris* (R Br) is the significant among the flora of academic section of Agriculture Development Trust, Malegaon. Milky latex of this plant is rich in terpenes. The *Syzygium cumini* (L) is the medicinal plant used to control the diabetes. Moreover, it contain insulin mimicking compounds. With this supposition, in addition to four plants in Table: 1, the *Astonia scholaris* (R Br) & *Syzygium cumini* (L) were also selected for the study. The tender stem pieces of *Vitis vinifera* (L) were collected from Baramati Agro Limited Pimpli (Tal – Baramati). *Santalum album* (L) & *Tectona grandis* (L) were procured from Govind Baug. *Lantana camera* (L); *Astonia scholaris* (R Br) & *Syzygium cumini* (L) were collected from greenery of Shardanagar. They were got

identified through experts of Botony Department & allowed for thoroughly drying at room temperature in shade. Dried stem pieces were then powdered using domestic electric mixture. Known quantity of each plant powder was soxhlet extracted with acetone for 24 hours. Each extract was evaporated to dryness. Weight of each extractive was noted. Based on preliminary study (Khyade, 2004 & Khyade *et al*., 2006), known quantity of each plant extract was dissolved in acetone to get desired concentrations (0.01 to 0.20 mg/ml for Vitis ; 0.15 to 0.32 mg/ml for Santalum; 0.20 to 0.38 mg/ml for Lantana; 0.30 to 0.50 mg/ml for teak; 0.1 to 0.30 mg/ml for Alstonia & 0.20 to 0.50 mg/ml for Syzygium). Camphene was used as a standard.Solutions of camphene (0.02-0.25 mg/ml) was prepared by dissolving appropriate quantity in known volume of acetone. The disease free layings (DFL) of polyvoltine, crossbreed race (PM x CSR_2) of silkworm, *Bombyx mori* (L) were procured from sericulture unit at the farm of Agriculture Development Trust, Malegaon (Baramati). They were processed for incubation through black boxing for 48 hours. The larvae were reared in laboratory condition on the leaves of mulberry (M-5 variety). Standard Methods of rearing (Krishnaswami, *et al*., 1978) were followed.

Soon after the fourth moult, the larvae of fifth instar were grouped into control (Untreated & acetone treated) groups and experimental groups (seven), each with fifty individuals. Ten microliters of each concentration of acetone extractives of Camphene (as a standard JHA); *Vitis vinifera* (L); *Santalum album* (L); *Lantana camera* (L); *Tectona grandis* (L); *Astonia shlolaris* (L) & *Syzygium cumini* (L) were topically applied with micropipette separately to the individual fifth instar larvae at 48 hours after the fourth moult. The larvae of all groups were maintained according to usual schedule. The chitin content of body wall was estimated at zero; 24; 48; 72; 96 and 120 hours after the fourth moult (from the first day to the fifth day of fifth instar). The method followed for chitin estimation was volumetric (Baishya & Hazarika, 1996; Vitthalrao Khyade, *et al*., 2006). Twenty larvae from each group were selected randomly and anaesthetized with little quantity of chloroform soaked cotton pad. They were dissected in insect saline (Yamaoka *et al*., 1971). The abdominal fat bodies & visceral organs were removed carefully.

FIGURE 1 : Pattern of chitin deposition in the body wall / integument at 120 hours after the fourth moult in silk worm, *Bombyx mori* (L)(Race: PM x CSR_2)recipient of acetone extractives of some non – mulberry plants.



After removing all the organ systems, trachae and adhering fat bodies the part remained was designated as integument (Jadhav & Kallapur, 1989). The integument of each larva was blotted & weighed on electronic balance. The integument piece of individual larva was placed in separate test tube containing 50 ml. of 30 percent potassium hydroxide (KOH) solution. All the test tubes in a group were placed in separate water bath. The contents of test tube were allowed for boiling for thirty minutes. After treating the integument with boiling potassium hydroxide solution, it was subsequently washed with distilled water; twice in 96% ethanol & twice in ether. Treated pieces of integument were weighed accurately on electronic balance. The weight of integument after

potassium hydroxide treatment corresponds to the quantity of chitin (mg/gm). Body wall chitin contents at zero and 120 hours after the fourth moult were considered as initial and final quantity of chitin respectively. Subtraction of initial quantity from final quantity give the quantity of chitin deposited in body wall of the fifth instar larvae for 120 hours after the fourth moult (zero to fifth days of fifth instar larvae). Quantity of chitin (mg/gm) deposited in the treated group was subtracted from the quantity of chitin deposited in the control group. The figure was divided by quantity of chitin deposited in control group. The quotient, thus obtained was multiplied by hundred to know percent reduction in the chitin in the integument of larvae of treated groups. The experiments were repeated for three times. Data was collected & subjected for statistical analysis (mean, standard deviation & student "t" test for knowing the significant level of treatment)(Norman & Baily, 1955). The figures of concentrations of acetone extractives were arranged on x- axis & that of percent reduction in chitin on y- axis. Dose response curve for each plant extractive was plotted (Fig. 1). The x- co-ordinate that corresponds to the value of fifty on y-axis in dose response curve was designated as ID_{50} value for given plant. Thus, ID50 value for each plant extractive was calculated through the use of respective dose response curve. The plot of dosages of acetone extractives of selected plants & percent change in the body wall chitin of larval instars of silkworm, *Bombyx mori* (L) is to be recognized as Punyamayee Dose Response Curve.

TABLE 2: Influence of acetone extractives of selected plants in the chitin content of body wall of fifth instar larvae of silkworm, *Bombyx mori* (L) (PM x CSR_2).

Plant	V. vinifera		A. scholaris		S. album		L. camera		S. cumini		T. grandis	
Sr.No.	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y
1.	0.00	16.895(±3.447) 0.000	0.00	16.895(±3.447) 0.000	0.00	16.895(±3.447) 0.000	0.000	16.895(±3.447) 0.000	0.000	16.895(±3.447) 0.000	0.24	16.895(±3.447) 0.000
2.	0.02	16.895(±3.786) 0.000	0.06	16.813(±3.438) 0.485	0.16	16.303(±3.872) 3.504	0.18	16.895(±4.376) 0.000	0.20	16.811(±3.912) 0.497	0.26	16.810(±4.913) 0.503
3.	0.04	16.811(±2.821) 0.497	0.08	16.472(±4.301) 2.503	0.18	15.627(±3.631) 7.977	0.20	16.557(±4.391) 2.000	0.22	16.726(±3.957) 1.000	0.28	16.642(±4.546) 1.497
4.	0.06	16.557(±3.634) 2.000	0.10	14.841(±3.664) 12.157	0.20	13.561(±3.293) 19.733	0.22	16.050(±3.876) 5.001	0.24	16.557(±3.916) 2.000	0.30	16.472(±3.021) 2.503
5.	0.08	15.959(±3.312) 5.540	0.12	11.644(±3.912) 31.080	0.22	10.365(±2.734) 38.650	0.24	15.458(±4.062) 8.505	0.26	16.303(±3.989) 3.503	0.32	16.134(±3.786) 4.504
6.	0.10	12.762(±3.649) 24.462	0.14	8.447(±2.953) 50.002	0.24	7.169(±1.378) 57.567	0.26	12.603(±3.751) 25.403	0.28	15.966(±4.063) 5.498	0.34	15.458(±3.412) 8.505
7.	0.12	9.735(±1.081) 42.379	0.16	5.251(±0.864) 68.919	0.26	3.979(±0.541) 76.448	0.28	9.406(±2.348) 44.326)	0.30	15.374(±3.907) 9.002	0.36	14.445(±3.594) 14.501
8.	0.14	4.801(±1.423) 71.583	0.18	2.054(±0.908) 87.842	0.28	1.942(±0.072) 88.505	0.30	6.209(±2.081) 63.249	0.32	14.529(±4.149) 14.004	0.38	12.283(±3.521) 27.298
9.	0.16	2.854(±0.172) 83.107	0.20	1.352(±0.523) 91.997	0.30	1.267(±0.069) 92.500	0.32	3.013(±0.876) 82.166	0.34	11.644(±3.248) 31.080	0.40	9.086(±1.485) 46.221
10.	0.18	1.689(±0.327) 84.084	0.22	0.844(±0.019) 95.004	0.32	0.761(±0.009) 95.495	0.34	1.605(±0.459) 90.500	0.36	8.447(±1.218) 50.002	0.42	5.891(±0.497) 65.131
11.	0.20	1.013(±0.249) 94.004	0.24	0.506(±0.037) 97.005	0.34	0.422(±0.003) 97.507	0.36	1.014(±0.019) 93.998	0.38	5.251(±0.823) 68.919	0.44	2.693(±0.628) 84.060
12.	0.22	0.506(±0.047) 97.005	0.26	0.338(±0.002) 97.999	0.36	0.421(±0.008) 97.508	0.38	0.591(±0.007) 96.501	0.40	2.054(±0.671) 87.842	0.46	1.267(±0.077) 92.500
13.	0.24	0.3379(±0.096) 98.00	0.28	0.338(±0.001) 97.999	0.38	0.421(±0.011) 97.508	0.40	0.379(±0.009) 97.756	0.42	0.845(±0.032) 94.998	0.48	0.591(±0.006) 96.501
14.	0.26	0.337(±0.091) 98.0	-	-	0.40	0.421(±0.017) 97.508	0.42	0.379(±0.002) 97.576	0.44	0.338(±0.011) 97.999	0.50	0.423(±0.008) 97.496
15.	0.28	0.337(±0.098) 98.0	-	-	-	-	0.44	0.379(±0.005) 97.756	0.46	0.338(±00.007) 97.999	0.52	0.423(±0.003) 97.496

X = conc. of acetone extractives (mg/ml).

Y = Body wall chitin (mg/gram).

- Each figure is the mean of three replications.
- Figures in parenthesis with + sign are the standard deviations.
- Figures below the quantity of chitin are the percent reduction over the control.

* = $P < 0.005$

* * = $P < 0.01$

* * * = $P < 0.001$

RESULT AND DISCUSSION

The body wall chitin(mg/ gm) deposited in the body wall of the fifth instars larvae at 0.00;48;72;96 and 120 hours after the fourth moult were found measured as: 6.841(+1.789);7.114(+0.786);9.512(+0.793);17.849(+1.437); 19.812(+1.437) and 23.736(+4.158) units respectively. The chitin deposition for 24, 48, 72, 96, and 12o hours after the fourth moult was calculated as: 0.273; 2.671; 11.008; 12.971 and 16.895 units respectively. Topical application of ten microlitres of camphene and various concentrations of acetone extractives of selected plants (*Vitis vinifera; Alstonia scholaris; Santalum album; Lantana camera; Syzyguim cumuni* and *Tectona grandis*) to the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR_2) at 48 hours after the fourth moult was found variously reflected into prolongation of larval life

and resulted into changes into in the chitin deposition in the body wall (integument). The chitin deposition in the body wall of larvae of untreated control group was found measured 16.895 mg/gm. Acetone solutions of camphene (0.02-0.06 mg/ml) was found observed into non-significant reduction in chitin deposition. The concentrations: 0.08-0.14mg/ml of camphene was enrolled significant reduction in chitin deposition. Percentage of chitin in the body wall of larvae treated with 0.08-0.14mg/ml 0f camphene is occupying the position 0n steeper part of the plot. Remaining concentrations of camphene (0.16-0.20 mg/ml) seems to be the most significant (they found to yield maximum possible reduction in chitin deposition). The lower concentrations of acetone extractives (0.02- 0.08 mg/ml) of *Vitis vinifera* were exhibited non significant reduction in the chitin content of larval integument. Significant reduction in chitin content of Vitis treated larvae was found in the group of 0.09 to 0.26 mg/ml acetone extractives. The most significant ($P<0.001$) reduction in the body wall chitin was observed in the group of larvae treated with 0.09, 0.13, 0.16, 0.18, 0.19 & 0.20 mg/ml acetone extractives of *Vitis vinifera*. The other concentrations of Vitis extractives exhibited significance of $P<0.05$ and $P<0.01$. The notable feature of groups of larvae treated with 0.16 to 0.26 mg/ml acetone extractives of Vitis was the appearance of non spinning larvae. The mature larvae in other groups of Vitis treatment (0.01 to 0.15 mg/ml) were found with normal spinning behavior. The concentrations (mg/ml) of acetone extractives of A*sltonia scholaris; Santalum album; Lantana camera; Syzygium cumini* & *Tectona grandis* which exhibited zero percent reduction in the body wall chitin of fifth instar larvae of silkworm, *Bombyx mori* (L) (Race : PM x CSR_2) were 0.01-0.05; 0.01-0.14; 0.01-0.17; 0.01-0.20 & 0.01-0.23 respectively. The concentrations (mg/ml) of acetone extractives of Alstonia ; Santalum ; Lantana ; Syzygium and Tectona, which were resulted into non – significant reduction in body wall chitin correspond to : 0.06 to 0.11; 0.15 to 0.19; 0.18 to 0.24; 0.21 to 0.27 & 0.24 to 0.33 mg/ml respectively.

During the early age (up to 48 hours) of fifth instar larvae of silkworm, *Bombyx mori* (L), the titer of juvenile hormone (JH) in the haemolymph is maintained at significant detectable level (Shi- Hong Gu & Yein Shing Chow, 1996). Rate of chitin deposition during this period seems to be non significant. Thereafter, the juvenile hormone (JH) in the larval haemolymph gets decreased rapidly. The most possible reason for this includes accelerative rate activity of esterase after 48 hours after the fourth moult (Ajami & Riddiford, 1973; Khyade, 2004). The present study demonstrate to decrease in chitin deposition in the body wall of fifth instar larvae of silkworm, *Bombyx mori* (L) (Race : PM x CSR_2) recipient of the exogenous juvenoid material in the form of acetone extractives of selected plants. The significant feature of exogenous juvenoids is to slow down the rate of chitin synthesis in the body of insects. The appreciable sclerotization before spinning seems to be prerequisite for metamorphosis to proceed (Omana Joy, 1984). The titer of juvenile hormone in the haemolymph of fifth instar larva in late age (last three days) is to be maintained at insignificant, undetectable level for the purpose to proceed metamorphosis through accelerate rate of metabolism including chitin deposition. Delay in the maturation for spinning in the larvae treated with juvenoids, as observed in the present study, may be to resume normal rate of chitin deposition.

The present study demonstrate the titer of exogenous juvenoid material get reflect into various conditions of juvenility (in the form of decreased amount of chitin in the body wall) of fifth instars larvae of silkworm, *Bombyx mori* (L) (Race : PM x CSR_2). Reduction in the deposition of chitin in body wall of treated larvae(irrespective of plant extractives and their concentrations too) recorded in the study, establish a positive effect, which seems to be in agreement with results obtained through the use of plant extractives and juvenoid compounds in silkworm larvae (Akai and Kobayashi,1971; Sharad Jagatap, 2007; Vitthalrao Khyade,2009). Selected doses of selected of plant extractives may be utilized for the purpose to sustain the larval age, which is essential to uplift the time required for eating mulberry leaves and amount of mulberry leaves eaten. If the maximum possible juvenoid effect in the form of reduction in body wall chitin in the fifth instar larvae of silkworm considered as hundred percent reduction in the chitin content, it has been found that, successive percent reduction from zero to hundred appear to be proportional to the topically applied concentration (dosage) within some narrow range (Fig. 1). The relationship between titer (concentration) of exogenous juvenoid material (acetone extractives of selected plants) & intensity of chitin deposition in the body wall of larvae appear to be in the form sigmoid curve, which, herewith entitled as Punyamayee Dose Response Curve. These curves seem to exhibit a characteristic S-form (sigmoid) displacement across the scale of concentration (mg/ml) of selected non mulberry plants. The change from zero to hundred percent effect commonly exhibited over 10-50 fold change in the dose topically applied. The concentrations (dosages) of acetone extractives of non mulberry plants in the study, on steeper slope of curves, seem to be most significant in the percent reduction in the body wall chitin. Therefore, the dosages of acetone extractives of plants on the steeper slope of Punyamayee Dose Response Curve may be called as effective dosages.

The effects of juvenoids involve inhibition of insect metamorphosis, significantly through reduction in chitin deposition (Slama, 1971). It has been proposed to express the concentration (dosage) of acetone extractives (Juvenoid) topically applied in terms of ID_{50} value. According to Slama, *et al*., (1974), the ID_{50} unit of juvenoid material (in microgram), which deposit fifty percent chitin in the body wall of insect larvae. The concentrations (mg/ml) of acetone extractives of non mulberry plants in the study, that inhibit the chitin deposition in the body wall of larvae by fifty percent can be calculated by the use of Punyamayee Dose Response Curves. Accordingly, the ID_{50} values of Camphene; *Vitis vinifera; Asltonia scholaris; Santalum album; Lantana camera; Syzygium cumini* & *Tectona grandis* were found calculated 0.100; 0.127;0.140;0.232;o.286;0.360 and 0.404 units (mg/ml) respectively. Ten microlitres out of thousand microlitres of each acetone solution was utilized for topical application on individual larva in each group. It

implies that, ten microliteres of each of acetone solution (Camphene; Vitis; Alstonia; Santalum; Lantan; Syzygium and Tectona) in the study correspond to:1.oo; 1.27;1.40;2.32;2.86;3.60; 4.04 micro gram units respectively. The Punyamayee Dose Response Curves in the study may form baseline platform for estimation of ID50 values of any compounds (plant derived; animal derived and synthetic compounds). The present study tried its best to establish preliminary work on screening the acetone extractives of selected plants for juvenoid activity in the fifth instar larvae of silkworm, *Bombyx mori* (L). (Race: PM x CSR_2). Farnasol Methyl Ether (FME) or acetone like solvents may serve the purpose to know intensity of juvenoids in any compound and plant or animal extractives. The Punyamayee Dose Response Curves may open a new avenue in the field of Juvenoid research.

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