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The monoterpene compounds for juvenile hormone activity through changes in pattern of chitin deposition in the integument of fifth instar larvae of silkworm, *Bombyx mori* (L) (PM x CSR2)

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ABSTRACT

The insects are a class of invertebrates within the arthropod phylum that have a chitinous exoskeleton. The leaf eating insects obtain their nutrients and growth promoting biocompounds from the variable or specific flora available for them. The plants on earth are the richest source of metabolites including juvenile hormone analogues for leaf eating insects like silkworm, *Bombyx mori* (L). Some of plant origin metabolites are acting as insects juvenoids for insect lives. They 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. The ten microliters of various concentrations of acetone solution of Fernasol Methyl Ether (FME) and each selected monoterpene compounds (Myrcene; Camphene; Cymene; Limonene and Eucalyptol) were used for topical application to individual larval instars of silkworm, *Bombyx mori* (L) (Race: PM x CSR2) at 48 hours after the fourth moult. The integument chitin of untreated control larvae; acetone treated control; FME treated larvae and monoterpene treated larvae was estimated at 120 hours after the fourth moult. Topical application of selected concentrations of acetone solutions of selected monoterpenes to fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2) was found reflected into the reduction in the deposition of chitin in the larval body wall. The

reduction in body wall chitin was found ranging from zero to hundred percent. The plot of concentrations of acetone solutions of FME and monoterepene compounds and percent reduction in the body wall chitin was found exhibiting a characteristic Sigmoid form of displacement, which herewith titled as "Punyamayee Baramati 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 ID50 value. The ID50 value of juvenoid contents of FME and selected monoterpene compounds can be defined as the specific unit (microgram), which enable to chitin to deposit fifty percent less in the body wall of larvae (In comparison with untreated control). Accordingly, the ID50 value calculated from the "Punyamayee Baramati Dose Response Curves" for FME was found measured 0.08 mg/ml. The ID50 values for monoterpene compounds: Myrcene; Camphene; Cymene; Limonene and Eucalyptol were found measured: 0.116; 0.122; 0.164; 0.172 and 0.208 mg/ml respectively. Acetone soluble juvenoid content of terpene compounds may be utilized efficiently for the fortified development of fifth instars of silkworm, *Bombyx mori* (L) and thereby, the cocoon quality. Sigmoid (S-form) "Baramati Dose Response Curve" may help for quantitative estimation of juvenoid contents of various terpene compounds and terpenoids.

***Keywords*:** FME; Terpenoids; Cymene Limonene; Phytophagus; juvenoids

1. INTRODUCTION

Humans regard certain insects as pests, and attempt to control them using insecticides and a host of other techniques. Some insects damage crops by feeding on sap, leaves or fruits. A few parasitic species are pathogenic. The study of phytophagous insects has been very important in agriculture. Probably from ancient times, humans have selected varieties of crop plants that are minimally attacked by insects, and in the last 100 years breeding programs have been important in specifically increasing plant resistance. They obtain their nutrients and growth promoting biocompounds from the variable or specific flora available for them. The plants on earth are the richest source of metabolites including juvenile hormone analogues for leaf eating insects like silkworm, *Bombyx mori* (L) (Vitthalrao B. Khyade, *et al* 2015).

Titers of the juvenile hormone (JH) & the moulting hormone (MH) serves a lot to orchestrate the progression of metamorphosis in the insects, like silkworm, *Bombyx mori* (L) The corpora allata belong to cephalic region of insect body secrete JH. Inhibition of morphogenetic programme at predetermined and group specific ontogenetic positions is the distinguishing feature of JH (Zaoral Slama, 1970). There are many compounds of plant derived, animal derived and synthetic that exhibit the biochemical properties of natural juvenile hormone of the insects. Such compounds are termed as "Juvenoids (Williams, 1956). Prolongation of larval age seems to be the significant influence of the exogenous topical application of acetone solutions of the juvenoids.

Further, the juvenoids are found plant material through suitable solvent exhibiting 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). The 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 & Kikichi (1998); Ratnasen (1988); Mamatha *et al* (1999) & Khyade (2002, 2003 & 2004).

Gopakumar, et al (1977) reported the juvenomimetic activity of extractives of some of the South Indian plants. This attempt leads to imagine the probability of occurrence of juvevenomimetic action in other plants. The larval instars of insects especially phytophagus, use to manage the titre of JH in their body and juvenoid contents received from the host plants. This is the prime requirement of phytophagous insects for metamorphosis to proceed. Sclerotized proteins and chitin contribute for rigidity of cuticle. This contribute for limited capacity for keeping the pace for the growth of insect body. For the purpose of growth and development to proceed, the body of insects replace the old cuticle through ecdysis or moulting. The process of ecdysis deserve periodicity and therefore, exert significant influence. The newly deposited cuticle contribute for nonsclerotized integument present below the older cuticle. This nascent integument exhibit strong furrows and have a capacity to expand during the process of ecdysis or moulting. The apolysis is the metamorphic event that initiates the ecdysis. Separation of epidermal cells from the older cuticle through the supportive action of moulting fluid and formation of ecdysial membrane are the significant features of apolysis.

Reynolds and Samuels (1996) reported the presence of enzymes, like protease and chitinase in the moulting fluid, integrated action of which is responsible for digestion of constituents of the older cuticle during the apolysis. It has been supposed that, shortly before ecdysis, the molting fluid, which has accumulated in the apolysial space, get reabsorbed. And this is for allowing the recycling of the individual constituents of older cuticle. The proteins of cuticle and chitin fibres through the apical membranes of epidermal cells, get secreted, which is responsible for opening the ecdysial space. Firstly, the proteins and chitin forms patches of cuticullin. This get followed by formation of so called the outer epicuticle. The procuticle get formed below the outer epicuticle.

The inner epicuticle get deposited and seals the epidermis. This seems essential for prevention of protection of cuticle from the digestive enzymes in moulting fluid. Before hardening or sclerotizatio of chitin, the body of insect get expand, which leads to release 9 or to shed) the older envelope in the form of exuvia. According to Carlson and Bentley (1977), the release of older cuticle during moulting in insects is through distinct motor programmes and through increasing body pressure. The behavior pertaining pre-ecdysis and ecdysis in insects are controlled by the action of moulting hormones, such as eclosion hormone. This eclosion hormone is secreted in response to falling the titre of ecdysteroid, which in it's turn causes the release of pre-ecdysis-triggering-hormone and ecdysis hormone (Truman and Riddiford, 1970; Kingan and Adams, 2000). The juvenile hormone and juvenoids regulate the quality of the moult (Ratnasen, 1988; Khyade, *et al*, 2003 and Khyade, 2004).

In the last larval stadium of holometabolous insects like silkworm, *Bombyx mori* (L), reduction in the titer of juvenile hormone (JH) in haemolymph is essential event for the initiation and metamorphosis and to change into the pupa (Mamatha, *et al*, 1999). Bioassay of activity of juvenile hormone and it's analogues (Juvenoid) have 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. 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).

The juvenoid activity of exogenous compounds is expressed in terms of units of percent reduction of chitin deposited in the body wall of larval stadia (Khyade, 2011 and Jagtap, 2014). It refers to the titre or dose or concentration of exogenous juvenoid compound topically applied responsible for percent inhibition of chitin deposition in the body wall of larval instars of insects, like silkworm, *Bombyx mori* (L). The terpenes are a large and diverse class of organic compounds, produced by a variety of plants. The terpenes are also produced by some insects such as termites or swallowtail butterflies, which emit terpenes from their osmeteria. They are often strong-smelling. They may protect the plants that produce them by deterring herbivores and by attracting predators and parasites of herbivores. The biochemical actions of natural insect juvenile hormone and terpenes and terpenoid compounds are similar. That is to say, the terpenes mimics the actions of natural "Insect Juvenile Hormone".

The difference between terpenes and terpenoids is that terpenes are hydrocarbons, whereas terpenoids contain additional functional groups. Screening the plant extractives for juvenoids seems to be well established attempt. To proceed on the same line, the present attempt on screening the acetone solution of selected terpene compounds has been planned.

Photo 1. *Bombyx mori* (L)

2. MATERIAL AND METHOD

The experimentation was divided into seven steps, which include: Rearing of larval instars of silkworm, *Bombyx mori* (L); Daily bioassay of body wall chitin of fifth instar larvae; Preparation of acetone solutions of selected monoterpenes; Grouping the fifth instar larvae and topical application of acetone solution of monoterpene; Bioassay of body wall chitin at 120 hours after the fourth moult; Statistical analysis of the data and Plotting the "Punyamayee Baramati Dose Response Curves" for the compounds used for topical application.

(A). Rearing of larval instars of silkworm, *Bombyx mori* (L):

The disease free layings (DFL) of polyvoltine, crossbreed race (PM x CSR2) 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 and Vitthalrao B. Khyade, 2004).

(B). Daily bioassay of body wall chitin of fifth instar larvae:

The chitin content of body wall was estimated at zero (soon after the fourth moult); 24; 48; 72; 96 and 120 hours after the fourth moult. The method followed for chitin estimation was volumetric (Baishya and Hazarika, 1996; Vitthalrao Khyade, *et al*, 2006). Twenty larvae for each time were selected randomly and anaesthetized with little quantity of chloroform soaked cotton pad. They were dissected in insect saline. The abdominal fat bodies and visceral organs were removed carefully. After removing all the organ systems, trachae and adhering fat bodies the part remained was designated as integument. The integument of each larva was blotted and 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; two times in ninety six percent ethanol and two times in ether. Treated pieces of integument (body wall) were weighed accurately on electronic balance. The weight of integument (body wall) after potassium hydroxide treatment corresponds to the quantity of chitin (mg/gm).

(C). Preparation of acetone solutions of selected monoterpenes:

The isoprene units contributes for a monoterpene, which have the molecular formula $C_{10}H_{16}$. With reference to chemical structure, the monoterpenes are either linear (acyclic) or contain rings. Biochemical changes such as oxidation or rearrangement are responsible to produce the related monoterpenoids. The monoterpenes compounds were selected for screening for their abilities of juvenoid activity in silkworm, *Bombyx mori* (L). Based on availability and suitability for the use, the monoterpene compounds selected in present attempt include: Myrcene; Camphene; Cymene; Limonene and Eucalyptol. The Myrcene, is also called as β-myrcene. It is an olefinic natural organic compound, which is classified more precisely as a monoterpene. The terpenes are dimers of isoprene, and myrcene is one of the most important. It is a component of the essential oil of several plants including bay,

cannabis, ylang-ylang, wild thyme, parsley, and hops. The Camphene is a bicyclic monoterpene, which is a minor constituent of many essential oils such as turpentine, cypress oil, camphor oil, citronella oil, neroli, ginger oil, and valerian. It is produced industrially by catalytic isomerization of the more common alpha-pinene. The Eucalyptol is a natural organic compound of a cyclic ether and a monoterpenoid class. The Cymene is a naturally occurring aromatic organic compound. It is classified as an alkylbenzene related to a monoterpene And the Limonene is monocyclic monoterpene compound belong to the constituent of citrus (plant family Rutaceae). Farnasol Methyl Ether (FME) was selected as standard "Insect Juvenoid Compound". All the monoterpene compounds (Myrcene; Camphene; Eucalyptol; Cymene and Limonene) were procured through the local chemical suppliers. Based on preliminary studies, known quantity of FME was dissolved in known volume of acetone so as to get desired concentration. Various concentrations of acetone solution of FME include: 00.010 to 00.160 mg/ml. Likewise, each monoterpene compound was dissolved in acetone to get desired concentrations (00.04 to 00.200 mg/ml for Myrcene; 00.06 to 0.210 mg/ml for Camphene; 00.100 to 0.250 mg/ml for Cymene; 00.10 to 0.260 mg/ml for Limonene; and 00.140 to 00.280 mg/ml for Eucalyptol). FME was used as a "standard Insect Juvenoid Compound" for comparison. Various concentrations (00.005 to 00.165 ppm) of FME were prepared by dissolving it's appropriate quantity in acetone.

(D). Grouping the fifth instar larvae and topical application of acetone solution of monoterpene:

Soon after the fourth moult, the larvae of fifth instar were grouped into control (Untreated and acetone treated, each one) groups and experimental groups (6 x 30), each with fifty individuals. Ten microliters of each concentration of acetone extractives of FME (as a standard Insect JHA); Myrcene; Camphene; Cymene; Limonene and Eucalyptol 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.

(E). Bioassay of body wall chitin at 120 hours after the fourth moult:

Body wall chitin contents of fifth instar larvae (Untreated Control group; Acetone Treated Control group and Monoterpene Treated groups) was carried out at 120 hours after the fourth moult. The method followed for chitin estimation was volumetric (Baishya and 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. The abdominal fat bodies and visceral organs were removed carefully. After removing all the organ systems, trachae and adhering fat bodies the part remained was designated as integument. The integument (body wall) of each larva was blotted and weighed on electronic balance. The integument (body wall) 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; two times in ninety six percent ethanol and two times 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).

(F). Statistical analysis of the data:

The experimentations were repeated for three times for the consistency in the results. Data was collected and subjected for statistical analysis (mean, standard deviation and student "t" test for knowing the significant level of treatment) (Norman and Baily, 1955). Soon after the fourth moult (zero hour) 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 (5 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. This 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.

(G). Plotting the "Punyamayee Baramati Dose Response Curves" for the compounds used for topical application:

Dose response curve for each compound was plotted (Fig. 1). The scale for plotting the graph, for X- axis was 1 = 00.010 mg/ml concentration of acetone solution. And that for Y-axis, the scale was 1 = 5 percent. Dose response curve for each compound 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 ID50 value for given compound. Thus, ID50 value for each compound in the study was calculated through the use of respective dose response curve. The plot of dosages of acetone extractives of selected compounds and percent change in the body wall chitin of larval instars of silkworm, *Bombyx mori* (L) is to be recognized as "Punyamayee Baramati Dose Response Curve".

3. RESULTS AND DISCUSSION

The results are summerised in Table 1 to 7. The amount of chitin(mg/ gm) deposited in the body wall of the fifth instar larvae at 0.00; 48; 72; 96 and 120 hours after the fourth moult were found measured as: 19.774 (±1.087); 19.779 (±1.143); 19.786 (±2.057); 20.679 (±1.789); 26.823 (±3.018) and 38.186 (±3.632) units respectively. In the untreated and acetone treated groups, the body wall chitin at 120 hours after the fourth moult was 38.186 (±3.632) and at 48 hours after the fourth moult was 19.786 (±2.057). Subtraction of chitin content at 48 from 120 hours gives the amount of chitin deposited during the experimental period (38.186 – 19.786 = 18.400). Topical application of ten microlitres of FME and selected monoterpenes was found reduction in chitin deposition in the body wall (integument). And the pattern was exhibiting significant response with reference to chitin deposition pattern in the body wall of fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2). The reduction in body wall chitin was found ranging from zero to hundred percent. The plot of concentrations of acetone solutions of FME and monoterepene compounds and percent reduction in the body wall chitin was found exhibiting a characteristic Sigmoid form of displacement, which herewith titled as "Punyamayee Baramati Dose Response Curve". The FME was found with lower concentration of it's acetone solution for reduction in chitin deposition in the body wall of fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

The concentrations namely, 00.000; 00.500; 01.00; 01.500; 02.000; 02.500; 03.000; 03.500; 04.000; 04.500 and 05.00 ppm (mg/ml) of FME were found with nonsignificant reduction in chitin deposition. The concentrations such as 05.400; 06.000; 07.000; 08.000; 09.000; 10.000 and 10.500 ppm (mg/ml) of FME were found with significant reduction in chitin deposition. Higher concentrations of FME (from 11.000 ppm and above) were with most significant reduction in the body wall chitin deposition (they found to yield maximum possible reduction in chitin deposition). The sigmoid curve of pattern of percent reduction in chitin deposition and concentrations of acetone solutions of FME and monoterpenes topically applied at 48 hours after the fourth moult to the larval instars of silkworm, *Bombyx mori* (L) (Race: PM x CSR2) in the study seems to reflect three groups of concentration of acetone solutions topically, which include: Nonsignificant; Significant and the most significant. The nonsignificant concentrations of acetone solutions of Myrcene; Camphene; Cymene; Limonene and Eucalyptol in the study include: 00.000 to 08.5; 00.000 to 09.000; 00.000 to 13.000; 00.000 to 13.500 and 00.000 to 17.500 ppm respectively. The significant concentrations of Myrcene; Camphene; Cymene; Limonene and Eucalyptol include: 09.000 to 14.500; 09.400 to 15.300; 13.500 to 19.500; 14.000 to 20.000 and 18.000 to 24.000 ppm respectively. That is to say, the percent reduction of chitin deposition of these concentrations occupy the steeper region of the sigmoid curve. The higher concentrations of acetone solutions of Myrcene; Camphene; Cymene; Limonene and Eucalyptol (15.000 and above; 15.500 and above; 20.000 and above; 20.500 and above; 24.500 and above respectively) resulted into the most significant reduction in the chitin deposition.

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. Rate of chitin deposition during this period seems to be non significant. Thereafter, the juvenile hormone (JH) in the larval haemolymph get decreased rapidly. The most possible reason for this include 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 slows 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 (1983). 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 FME and terpenes (let us label them "Silkworm 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 instar 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 acetone solution of FME and monoterpenes; 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 Juvenoids compounds in silkworm larvae (Akai and Kobayashi, 1971; Sharad Jagatap, 2007; Vitthalrao Khyade, 2009). Selected doses of selected of monoterpenes 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.

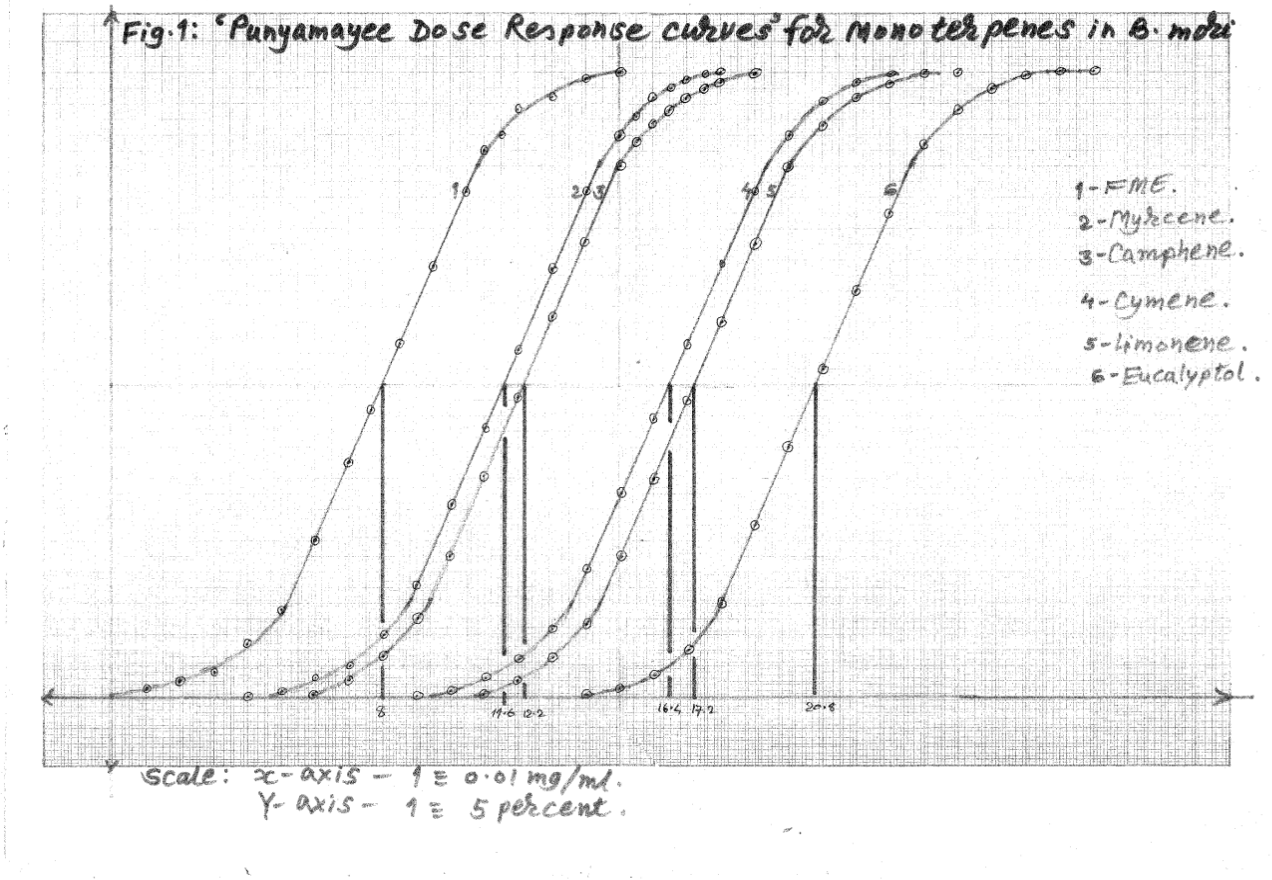

Fig. 1. "Punyamayee Baramati" Dose Response Curve for Monoterpenes in Silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

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. The relationship between titer (concentration) of exogenous juvenoid material (acetone solutions of selected FME and monoterpenes) & intensity of chitin deposition in the body wall of larvae appear to be in the form sigmoid curve, which, herewith entitled as "Punyamayee Baramati Dose Response Curve". These curves seems to exhibit a characteristic S-form (sigmoid) displacement across the scale of concentration (mg/ml) of FME and monoterpenes. 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 solutions of FME and monoterpenes in the study, on steeper slope of curves, seems to be most significant in the percent reduction in the body wall chitin. Therefore, the dosages of acetone solutions of FME and monoterpenes on the steeper slope of "Punyamayee Baramati 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 (1974). 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.

Table 1. Chitin content in the body wall of the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

Serial No.	Hour After the Fourth Moult	Body Wall Chitin Content (mg/Gm)
1	000.000	19.774 (± 1.087)
2	024.000	19.779 (± 1.143)
3	048.000	19.786 (± 2.057)
4	072.000	20.679 (± 1.789)
5	096.000	26.823 (± 3.018)
6	120.000	38.186 (± 3.632)

- Each figure is the mean of three replications.
- Figures with ± sign in parentheses are the standard deviations.
- Chitin Deposition for Untreated Control Larvae = Chitin content at 120 hours after the fourth moult – Chitin content at 48 hours after the fourth moult (18.4 = 38.186 – 19.786).

Table 2. Chitin content of the body wall of the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2) recipient of topical application of various concentration of acetone solution of Fernasol Methyl Ether (FME) at 48 hours after the fourth moult.

X	Concentration of Acetone Solution (ppm)	Body Wall Chitin (mg / gm)	Chitin Deposition (mg / gm)	Percent Reduction	Y
00.000	00.000	38.186 (± 4.673)	18.400	000.000	000.000
00.500	00.005	38.002 * (± 4.651)	18.216	01.000	000.200
01.000	00.010	37.910 * (± 4.397)	18.124	01.500	000.300
01.500	00.015	37.823* (± 4.089)	18.037	02.000	000.400

02.000	00.020	37.726* (± 3.391)	17.940	02.5000	000.500
02.500	00.025	37.634* (± 3.906)	17.848	03.000	000.600
03.000	00.030	37.542* (± 4.289)	17.756	03.500	000.700
03.500	00.035	37.266* (± 3.258)	17.483	05.000	001.000
04.000	00.040	36.990 * (± 4.078)	17.204	06.500	01.300
04.500	00.045	36.346 * (± 3.966)	16.560	10.000	02.000
05.000	00.050	35.610* (± 4.023)	15.824	14.000	02.800
05.400	00.054	34.966* * (± 3.843)	15.180	17.500	03.500
06.000	00.060	35.586* * (± 4.143)	13.800	25.000	05.000
07.000	00.070	31.286 * * (± 4.518)	11.500	37.500	07.000
08.000	00.080	28.986 * * (± 3.513)	09.200	50.000	10.000
09.000	00.090	26.686* * (± 3.795)	06.900	62.500	12.500
10.000	00.100	24.386* * (± 3.786)	04.600	75.000	15.000
10.500	00.105	23.236* * (± 3.897)	03.450	81.250	16.250
11.000	00.110	22.362* * * (± 3.841)	02.576	86.000	17.200
11.500	00.115	21.718* * * (± 4.948)	01.932	89.500	17.900
12.000	00.120	21.258* * * (± 4.013)	01.472	92.000	18.400
12.500	00.125	20.798* * * (± 3.427)	01.012	94.500	18.900
13.000	00.130	20.522* * * (± 3.734)	00.736	96.000	19.200
13.500	00.135	20.246* * * (± 3.964)	00.460	97.000	19.500
14.000	00.140	20.062* * * (± 3.687)	00.276	98.500	19.700
14.500	00.145	19.878* * * (± 3.789)	00.092	99.500	19.900
15.000	00.150	19.786 * * *	00.000	100.00	20.000

		(± 3.881)			
15.500	00.155	19.786 * * * (± 3.963)	00.000	100.00	20.000
16.000	00.160	19.786* * * (± 3.794)	00.000	100.000	20.000

1. Each figure is the mean of three replications;
2. Figures in parenthesis with ± sign are the standard deviations.
3. * = P < 0.005 ; * * = P < 0.01 And * * * = P < 0.001

Table 3. Chitin content of the body wall of the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2) recipient of topical application of various concentration of acetone solution of Myrcene (Monoterpene) at 48 hours after the fourth moult.

X	Concentration of Acetone Solution (ppm)	Body Wall Chitin (mg / gm)	Chitin Deposition (mg / gm)	Percent Reduction	Y
04.000	00.040	38.186* (± 4.729)	18.400	00.000	00.000
04.500	00.045	38.186* (± 4.337)	18.400	00.000	00.000
05.000	00.050	38.094* (± 3.899)	18.308	00.500	00.100
05.500	00.055	38.002* (± 4.107)	18.216	01.000	00.200
06.000	00.060	37.818 * (± 4.786)	18.032	02.000	00.400
06.500	00.065	37.726* (± 4.517)	17.940	02.500	00.500
07.000	00.070	37.358* (± 3.583)	17.572	04.500	00.900
07.500	00.075	36.898* (± 4.404)	17.112	07.000	01.400
08.000	00.080	36.438* (± 3.651)	16.652	09.500	01.900
08.500	00.085	35.794* (± 3.793)	16.008	13.000	02.500
09.000	00.090	34.966* * (± 4.761)	15.180	17.500	03.500
10.000	00.100	32.666* * (± 4.583)	12.880	30.000	06.000

11.000	00.110	30.3666* * (± 4.188)	10.580	42.500	08.500
12.000	00.120	28.066* * (± 3.919)	08.280	55.000	11.000
13.000	00.130	25.766* * (± 4.724)	05.980	67.500	13.500
14.000	00.140	23.466* * (± 4.592)	03.680	80.000	16.000
14.500	00.145	22.316* * (± 3.798)	02.530	86.250	17.250
15.000	00150	21.718* * * (± 4.478)	01.932	89.500	17.900
15.500	00.155	21.074 (± 4.076)	01.288	93.000	18.600
16.000	00.160	20.706* * * (± 3.877)	00.920	95.000	19.000
16.500	00.165	20.430* * * (± 3.813)	00.644	96.500	19.300
17.000	00.170	20.246* * * (± 3.845)	00.460	97.500	19.500
17.500	00.175	20.154* * * (± 2.892)	00.368	98.000	19.600
18.000	00.180	20.062* * * (± 2.883)	00.276	98.500	19.700
18.500	00.185	19.878* * * (± 4.729)	00.092	99.500	19.900
19.000	00.190	19.786 * * * (± 3.071)	00.000	100.00	20.000
19.500	00.195	19.786* * * (± 2.984)	00.000	100.00	20.000
20.000	00.200	19.786 * * * (± 3.715)	00.000	100.00	20.000
20.500	00.205	19.786* * * (± 2.946)	00.000	100.00	20.000
21.000	00.210	19.786* * * (± 3.246)	00.000	100.000	20.000
21.500	00.215	19.786* * * (± 3.351)	00.000	100.000	20.000

1. Each figure is the mean of three replications;
2. Figures in parenthesis with ± sign are the standard deviations.
3. * = P < 0.005 ; * * = P < 0.01 And * * * = P < 0.001

Table 4. Chitin content of the body wall of the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2) recipient of topical application of various concentration of acetone solution of Camphene (Monoterpene) at 48 hours after the fourth moult.

X	Concentration of Acetone Solution (ppm)	Body Wall Chitin (mg / gm)	Chitin Deposition (mg / gm)	Percent Reduction	Y
05.000	00.050	38.186* (± 4.786)	18.400	00.000	00.000
05.500	00.055	38.186* (± 4.758)	18.400	00.000	00.000
06.000	00.060	38.186* (± 4.877)	18.400	00.000	00.000
06.500	00.065	38.002* (± 4.647)	18.216	01.000	00.200
07.000	00.070	37.818* (± 4.673)	18.032	02.000	00.400
07.500	00.075	37.450* (± 4.696)	17.664	04.000	00.800
08.000	00.080	37.082* (± 3.756)	17.296	06.000	01.200
08.500	00.085	36.530 * (± 3.938)	16.744	09.000	01.800
09.000	00.090	35.978* (± 4.088)	16.192	12.000	02.400
09.400	00.094	35.426* * (± 4.413)	15.640	15.000	03.000
10.000	00.100	34.046 (± 3.836)	14.260	22.500	04.500
11.000	00.110	31.746* * (± 4.273)	11.960	35.000	07.000
12.000	00.120	29.446* * (± 3.781)	09.660	47.500	09.500
13.000	00.130	27.146* * (± 4.024)	07.360	60.000	12.000
14.000	00.140	24.846* * (± 3.791)	05.060	72.000	14.500
15.000	00.150	22.546* * (± 4.333)	02.760	85.000	17.000
15.300	00.153	21.856* * (± 4.526)	02.070	88.750	17.75
15.500	00.155	21.626* * * (± 3.589)	01.840	90.000	18.000
16.000	00.160	21.166* * * (± 3.019)	01.380	92.500	18.500

16.500	00.165	20.890* * * (± 3.326)	01.104	94.000	18.800
17.000	00.170	20.614* * * (± 3.089)	00.828	95.500	19.100
17.500	00.175	20.430* * * (± 3.581)	00.644	96.500	19.300
18.000	00.180	20.246* * * (± 3.334)	00.460	97.500	19.500
18.500	00.185	20.062* * * (± 2.789)	00.276	98.500	19.700
19.000	00.190	19.970* * * (± 3.061)	00.184	99.000	19.800
19.500	00.195	19.878* * * (± 2.926)	00.092	99.500	19.900
20.000	00.200	19.786* * * (± 2.911)	00.000	100.00	20.000
20.500	00.205	19.786* * * (± 3.091)	00.000	100.00	20.000
21.000	00.210	19.786* * * (± 2.517)	00.000	100.00	20.000
21.500	00.215	19.786* * * (± 2.645)	00.000	100.00	20.000
22.000	00.220	19.786* * * (± 2.853)	00.000	100.00	20.000

1. Each figure is the mean of three replications;
2. Figures in parenthesis with ± sign are the standard deviations.
3. * = P < 0.005 ; * * = P < 0.01 And * * * = P < 0.001

Table 5. Chitin content of the body wall of the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2) recipient of topical application of various concentration of acetone solution of Cymene (Monoterpene) at 48 hours after the fourth moult.

X	**Concentration of Acetone Solution (ppm)**	**Body Wall Chitin (mg / gm)**	**Chitin Deposition (mg / gm)**	**Percent Reduction**	**Y**
09.500	00.095	38.186* (± 4.817)	18.400	00.000	00.000
10.000	00.100	38.186* (± 4.801)	18.400	00.000	00.000
10.500	00.105	37.910* (± 4.678)	18.124	01.500	00.500

11.000	00.110	37.726* (± 4.732)	17.940	02.500	00.800
11.500	00.115	37.450* (± 4.789)	17.664	04.000	01.200
12.000	00.120	37.082* (± 4.757)	17.296	06.000	01.200
12.500	00.125	36.806* (± 5.842)	12.020	07.500	01.500
13.000	00.130	36.254* (± 4.863)	16.468	10.500	02.100
13.500	00.135	35.656* * (± 5.781)	15.870	13.750	02.750
14.000	00.140	34.506* * (± 5.291)	14.720	20.000	04.000
15.000	00.150	32.206* * (± 5.045)	12.420	32.500	06.500
16.000	00.160	29.906* * (± 4.893)	10.120	45.000	09.000
17.000	00.170	27.606* * (± 4.923)	07.820	57.500	11.500
18.000	00.180	25.306* * (± 4.811)	05.520	70.000	14.000
19.000	00.190	23.006* * (± 4.845)	03.220	82.500	16.500
19.500	00.195	21.856* * (± 4.759)	02.070	88.750	17.750
20.000	00.200	21.258* * * (± 3.813)	01.472	92.000	18.400
20.500	00.205	20.798* * * (± 4.321)	01.012	94.500	18.900
21.000	00.210	20.430* * * (± 3.062)	00.644	96.500	19.300
21.500	00.215	20.246* * * (± 4.562)	00.460	97.500	19.500
22.000	00.220	20.154* * * (± 4.181)	00.368	98.000	19.600
22.500	00.225	20.062* * * (± 4.393)	00.276	98.500	19.700
23.000	00.230	19.970* * * (± 3.678)	00.184	99.000	19.800

23.500	00.235	19.878* * * (± 4.639)	00.092	99.500	19.900
24.000	00.240	19.786* * * (± 4.223)	00.000	100.00	20.000
24.500	00.245	19.786* * * (± 4.514)	00.000	100.00	20.000
25.000	00.250	19.786* * * (± 3.559)	00.000	100.00	20.000
25.500	00.255	19.786 (± 4.035)	00.000	100.00	20.000
26.000	00.260	19.786* * * (± 4.418)	00.000	100.00	20.000

1. Each figure is the mean of three replications;
2. Figures in parenthesis with ± sign are the standard deviations.
3. * = P < 0.005 ; * * = P < 0.01 And * * * = P < 0.001

Table 6. Chitin content of the body wall of the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2) recipient of topical application of various concentration of acetone solution of Limonene (Monoterpene) at 48 hours after the fourth moult.

X	**Concentration of Acetone Solution (ppm)**	**Body Wall Chitin (mg / gm)**	**Chitin Deposition (mg / gm)**	**Percent Reduction**	**Y**
09.500	00.095	38.186* (± 5.093)	18.400	00.000	00.000
10.000	00.100	38.186* (± 4.956)	18.400	00.000	00.000
10.500	00.105	38.186* (± 5.789)	18.400	00.000	00.000
11.000	00.110	38.094* (± 4.886)	18.308	00.500	00.100
11.500	00.115	38.002* (± 4.857)	18.216	01.000	00.200
12.000	00.120	37.818* (± 5.856)	18.032	02.000	00.400
12.500	00.125	37.634* (± 4.832)	17.848	03.000	00.600
13.000	00.130	37.266* (± 5.847)	17.48	05.000	01.000
13.500	00.135	36.806* (± 5.165)	17.020	07.500	01.500

14.000	00.140	36.346* * (± 4.817)	16.560	10.000	02.000
15.000	00.150	38.046* * (± 4.858)	14.260	22.500	04.500
16.000	00.160	31.746* * (± 4.991)	11.960	35.000	07.000
17.000	00.170	29.446* * (± 4.924)	09.660	47.000	09.500
18.000	00.180	27.146* * (± 4.817)	07.360	60.000	12.000
19.000	00.190	24.846* * (± 4.817)	05.060	72.000	14.500
20.000	00.200	22.546* * (± 5.871)	02.760	85.000	17.000
20.500	00.205	21.626* * * (± 5.371)	01.840	90.000	18.000
21.000	00.210	21.166* * * (± 4.897)	01.380	92.500	18.500
21.500	00.215	20.798* * * (± 3.367)	01.012	94.500	18.900
22.000	00.220	20.614* * * (± 5.093)	00.828	95.500	19.100
22.500	00.225	20.430* * * (± 4.951)	00.644	96.500	19.300
23.000	00.230	20.246* * * (± 3.897)	00.460	97.500	19.500
23.500	00.235	20.154* * * (± 4.556)	00.368	98.000	19.600
24.000	00.240	20.062* * * (± 3.896)	00.276	98.500	19.700
24.500	00.245	19.970* * * (± 4.226)	00.184	99.000	19.800
25.000	00.250	19.878* * * (± 4.521)	00.092	99.500	19.900
25.500	00.255	19.786* * * (± 4.669)	00.000	100.00	20.000
26.000	00.260	19.786* * * (± 3.997)	00.000	100.000	20.000
26.500	00.265	19.786* * * (± 3.613)	00.000	100.000	20.000

1. Each figure is the mean of three replications;
2. Figures in parenthesis with ± sign are the standard deviations.
3. * = P < 0.005 ; * * = P < 0.01 And * * * = P < 0.001

Table 7. Chitin content of the body wall of the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2) recipient of topical application of various concentration of acetone solution of Eucalyptol (Monoterpene) at 48 hours after the fourth moult.

X	Concentration of Acetone Solution (ppm)	Body Wall Chitin (mg / gm)	Chitin Deposition (mg / gm)	Percent Reduction	Y
14.000	00.140	38.186* (± 4.951)	18.400	00.000	00.000
14.500	00.145	38.186* (± 5.033)	18.400	00.000	00.000
15.000	00.150	38.002* (± 4.487)	18.216	01.000	00.200
15.500	00.155	37.818* (± 5.112)	18.032	02.000	00.400
16.000	00.160	37.532* (± 4.982)	17.756	03.500	00.700
16.500	00.165	37.266* (± 5.891)	17.480	05.000	01.000
17.000	00.170	36.806* (± 5.278)	17.020	07.500	01.500
17.500	00.175	36.162* (± 5.294)	16.376	11.000	02.200
18.000	00.180	35.426* * (± 4.686)	15.640	15.000	03.000
19.000	00.190	33.126* * (± 4.187)	13.340	27.500	05.500
20.000	00.200	30.826* * (± 4.436)	11.040	40.000	08.000
21.000	00.210	28.526* * (± 5.873)	08.740	52.500	10.500
22.000	00.220	26.226* * (± 4.764)	06.440	65.000	13.000
23.000	00.239	23.926* * (± 4.928)	04.140	77.500	15.500
24.000	00.240	21.626* * (± 4.193)	01.840	90.000	18.000
24.500	00.245	20.890* * * (± 4.826)	01.104	94.000	18.800

25.000	00.250	20.522* * * (± 4.959)	00.736	96.000	19.200
25.500	00.255	20.246* * * (± 5.294)	00.460	97.500	19.500
26.000	00.260	20.062* * * (± 3.393)	00.276	98.500	19.700
26.500	00.265	19.970* * * (± 3.748)	00.184	99.000	19.800
27.000	00.270	19.878* * * (± 5.614)	00.092	99.500	19.900
27.500	00.275	19.786* * * (± 3.789)	00.000	100.00	20.000
28.000	00.280	19.786* * * (± 4.441)	00.000	100.00	20.000
28.500	00.285	19.786* * * (± 5.136)	00.000	100.000	20.000
29.000	00.290	19.786* * * (± 4.297)	00.000	100.000	20.000

1. Each figure is the mean of three replications;
2. Figures in parenthesis with ± sign are the standard deviations.
3. * = P < 0.005 ; * * = P < 0.01 And * * * = P < 0.001

4. CONCLUSIONS

The concentrations (mg/ml) of acetone solutions of FME and monoterpenes 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 Baramati Dose Response Curves". Accordingly, the ID_{50} values for FME; Myrcene; Camphene; Cymene; Limonene and Eucalyptol were found calculated 00.080; 00.116; 00.122; 00.164; 00.172 and 00.208 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.

The "Punyamayee Baramati 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 solutions of FME and selected monoterpenes for juvenoid activity in the fifth instar larvae of silkworm, *Bomby xmori* (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. The monoterpenes deserve many more cellular and molecular activities that could potentially underlie their juvenomimetic index with reference to the phytophagous insects like, silkworm, *Bombyx mori* (L). The present attempt is going to help to establish maximum tolerated dose of monoterpene to be used for future trials in which the efficacy of monoterpenes will be tested for qualitative improvement of silk spinned by mature fifth instar

larvae of silkworm, *Bombyx mori* (L). If the efficacy is seen in larval developmental setting, it will likely trigger future development and testing the monoterpenes for the fortified health of larval instars, that could spin the qualitative silky cocoon. The monoterpenes are thus an example of the development of agents that will bridge the areas of sericulture. The Baramati attempt of use of terpenes for topical application to the larval instars of silkworm, *Bombyx mori* (L) hope more efficiently benefitting the areas of both the areas of sericulture and juvenoid research. And the "Punyamayee Baramati Dose Response Curves" in the present attempt may open a new avenue in the field of Juvenoid research.

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References

[1] Ajami, A. M. and Riddiford, L. M. (1973). Comparative metabolism of the cecropia juvenile hormone. *J. Insect Physiol.* 19: 635-646.

[2] Baishya, R. L. and Hazarika, L. R. (1996). Effect of methoprene and diflubenzuron on water, lipid, protein and chitin of Dicladispa armigera (Coleoptera: Chrysomelidae). *Entomon,* 21 91(1): 7-11.

[3] Calvez, B., Hirn, M. and Reggi, M. (1976). Progress of development programme during the last larval instar of Bombyx mori (L). Relationship with food intake, ecdyosteroids and juvenile hormone. *Journal of Insect Physiology,* 24(4): 233-239.

[4] Gopakumar B., Ambika, B. and Prabhu, V. K. K. (1977). Juvenmimetic activity in some south Indian plants and their probable cause of this activity in Morus alba (L). *Entomon,* 2: 259-261.

[5] Grenier and Grenier (1983). Fenoxycarb, a fairlynew growth regulator: a review of its effects on insects. *Ann. App. Biol.* 122: 369-403.

[6] Jadhav G. and Kallapur V. L. (1989). Contribution of tissue protein to the cocoon shell in the fifth instar silk worm, Bombyx mori (L).

[7] Kamimura M. and Kiguchi M. (1980). Effect of juvenile hormone analogue on fifth stadium larvae of silk worm, Bombyx mori (L) (Lepidoptera: Bombycidae). *Appl. Entomol. Zool.* 33(2): 333-338.

[8] Khyade, V. B., Patil, S. B., Khyade, S. V. and Bhawane G. P. (2002). Influence of acetone maceratives of Vitis vinifera on the larval parameters of silk worm, Bombyx mori (L). *Indian Journal of Comparative Animal Physiology,* 20: 14-18.

[9] Khyade V. B., Patil, S. B., Khyade, S. V. and Bhawane, G. P. (2003). Influence of acetone maceratives of Vitis vinifera on the economic parameters of silk worm, Bombyx mori (L). *Indian Journal of Comparative Animal Physiology*. 21: 28-32.

[10] Khyade V. B. (2004). Influence of juvenoids on silk worm, Bombyx mori (L). Ph.D. Thesis, Shivaji University, Kolhapur, India.

[11] Khyade, V. B. and Ganga V. Mhamane (2005). Vividh Vanaspati Arkancha Tuti Reshim Kitak Sangopanasathi Upyojana. Krishi Vdnyan. 4, 18-22.

[12] Khyade, V. B.; Poonam B. Patil; M. Jaybhay; Rasika R. Gaikwad; Ghantaloo, U. S.; Vandana D. Shinde; Kavita H. Nimbalkar and Sarwade, J. P. (2007). Use of digoxin for improvement of economic parameters in silk worm, Bombyx mori (L). Bioinfrmatics (Zoological Society of India).

[13] Krishnaswami, S., Narasimhana, M. N., Suryanarayana, S. K. and Kumaraj, S. (1978). Sericulture Manual – ll: Silk worm Rearing. F A O , United Nation's Rome: 131.

[14] Mamatha, D. N., Nagalakshmma, K. and Rajeshwara Rao, M. (1999). Impact of selected Juvenile Hormone Mimics on the organic constituents of silk worm, Bombyx mori (L).

[15] Norman, T. J. and Baily (1955). Statistical Methods.

[16] Omana Joy and Shyamala, M. B. (1983). Non-spinning syndrome in silk worm: Occurrence and Pathology. Research paper presented for National Seminar on silk research and development, Banglore, India.

[17] Prabhu, V. K. K., John, M. and Ambika, B. (1973). Juvenile hormone activity in some south Indian plants. *Current Science,* 42: 72-726.

[18] Ratnasen (1988). How does juvenile hormone cause more silk yield. *Indian Silk:* 21-22.

[19] Riddiford, L. M. (1985). Hormone action at cellular level. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology. G. A. Kerkut and L. I. Gilbert (Eds.). 8: 37-64. (Pergamon press, Oxford).

[20] Riddiford, L. M. (1994). Cellular and molecular actions of Juvenile hormone: General consideration and premetamorphic actions. *J. Adv. Insect Physiology*. 24: 213-214.

[21] Sehnal F. and Rambold, H. (1985). Brain stimulation and juvenile hormone production in insect larvae. *Experentia.* 44: 684-685.

[22] Slama, K. (1969). Plant as source material with insect hormone activity. *Ent. Exp. Appl.* 12: 721-728.

[23] Slama, K. (1971). Insect juvenile hormone analogues. *Ann. Rev. Biochem.* 40: 1079-1102.

[24] Slama, K., Romanuk, M. and Sorm, F. (1974). Insect hormones and Bioanalogues. Springer Verlag, Wein and New York.

[25] Slama, K. (1985). Pharmacology of Insect Juvenile Hormones. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology. (Eds. G. A. Kerkut and L. I. Gilbert). Vol. 11: 357-394. Pergamon Press, Oxford, New York.

[26] Slama, K. (1979). Insect Hormones and anti-hormones in plants. Herbivores, their interaction with secondary plant metabolites. (Eds. G. A . Rosenthal and D. H. Janzen; Academic Press, New York): 683-700.

[27] Williams, C. M. (1956). The Juvenile Hormone of Insects. *Nature.* 178: 212-213.

[28] Zaoral, M. and Slama, K. (1970). Peptides with juvenile hormone activity. *Science.* 170: 92-93.

[29] Jagtap, Sharad G. (2007). Effect of plant juvenoids on consumption and utilization of mulberry leaves by silkworm, Bombyx mori (L). M. Phil. Dissertation, Alagappa University, Karaikudi - 630003 (India).

[30] Vitthalrao B. Khyade and Karel Slama (2015). Screening of acetone solution of FME and Selected Monoterpene Compounds for Juvenile Hormone Activity Through Changes in pattern of Chitin Deposition in the Integument of Fifth instar larvae of silkworm, Bombyx mori (L) (PM x CSR2). *IJBRITISH*, 2(3) (2015) 68-90.

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